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(54) Title: METHODS AND COMPOSITIONS RELATED TO STAUFEN 1 BINDING SITES FORMED BY DUPLEXING ALU ELEMENTS

(57) Abstract: Disclosed are compositions and methods for identifying binding sites of targets of Stau1-mediated mRNA decay; methods and compositions for treating subjects with conditions resulting from Stau1-mediated mRNA decay, and method of screening for therapeutic agents. Also disclosed is the new pathway as a means for cells to down-regulate the expression of Stau1-binding mRNAs.



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METHODS AND COMPOSITIONS RELATED TO STAUFEN 1 BINDING SITES FORMED BY DUPLEXING ALU ELEMENTS

1. This Application claims the benefit of U.S. Provisional Application No. 61/440,967, filed on February 9, 2011, which is incorporated by reference herein in its entirety.

2. This invention was made with government support under National Institutes of Health Grant GM074593, R01 GM074593, NCI T32 CA09363, 1S10 RR026501, 1S10 RR027241, NIH NIAID P30 AI078498, NIH/NCRR RR-01646, and NSF award DMR-0225180. The government has certain rights in the invention.

I. BACKGROUND OF THE INVENTION

3. Expression of truncated or nonsense proteins play a role or are the causative agent in many inherited disorders and cancers. These truncated or nonsense proteins result from mutations or aberrant mRNA splicing that result in early termination signals in the mRNA. Mammalian Staufen1 (Stau1) is an RNA binding protein that binds to extensive RNA secondary structures, primarily through one or more double-stranded RNA-binding domains. In mammals, the Stau1 gene is ubiquitously expressed and is involved in mRNA transport and translational control. Stau1 is associated with degradation of translationally active mRNAs that bind the double-stranded (ds)RNA binding protein STAU1, a process known as Stau1-mediated mRNA decay or SMD. What is needed are methods of identifying targets for SMD and compositions that can modulate SMD so that disorders associated with early termination signal in mRNA can be properly identified and treated.

II. SUMMARY OF THE INVENTION

4. In accordance with the purposes of this invention, as embodied and broadly described herein, this invention, in one aspect, relates to methods of identifying targets for Staufen 1 (Stau1) mediated decay (SMD); screening for agents that modulate SMD and treating subjects with conditions that result from or modified by affecting SMD.

5. Additional advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice of the invention. The advantages of the invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

III. BRIEF DESCRIPTION OF THE DRAWINGS

6. The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several embodiments of the invention and together with the description, serve to explain the principles of the invention.

5 7. Figure 1 shows that 11 of 12 transcripts that were upregulated in human cells depleted of Stau1 using three independently performed microarray analysis are upregulated using RT-PCR and transcript-specific primers. Notably, RNAs analyzed were identical to the RNAs from Control and Stau1 siRNA-treated samples analyzed in Figure 2. The level of each test transcript was normalized to the level of SMG7 mRNA, which is insensitive to Stau1
10 siRNA and served to control for variations in RNA recovery. Numbers below each lane specify the fold change in the level of each test transcript in cells treated with Stau siRNA relative to Control siRNA, the latter of which was defined as 1.

8. Figure 2 shows that c-JUN, SERPINE1 and IL7R mRNAs are increased in abundance in human cells depleted of either Upf1 or Stau1 but not Upf2. HeLa cells were
15 transiently transfected with Stau1, Stau1(A), Upf1, Upf1(A) or Upf2 siRNA or, to control for nonspecific depletion, Control siRNA. Three days later, protein and RNA were purified. Figure 2A shows Western blot analysis, where the level of Vimentin serves to control for variations in protein loading, and the normal level of Stau1, Upf1 or Upf2 is determined in the presence of Control siRNA. Figure 2B shows RT-PCR analysis of the level of endogenous c-
20 JUN mRNA (upper), SERPINE1 mRNA or IL7R mRNA (lower), each of which is normalized to the level of endogenous SMG7 mRNA. The normalized level of each mRNA in the presence of Control siRNA is defined as 1. RT-PCR results are representative of three independently performed experiments that did not differ by the amount specified.

9. Figure 3 shows that lncRNA_AF087999 ($\frac{1}{2}$ -sbsRNA1) binds to and reduces the
25 abundance of specific SMD targets. Figure 3a shows predicted base-pairing between the Alu element within the SERPINE1 or FLJ21870 3'UTR and the Alu element within $\frac{1}{2}$ -sbsRNA1, where 1 was defined as the first transcribed nucleotide of each mRNA or $\frac{1}{2}$ -sbsRNA1(S). Figure 3b shows at left: Western blotting (WB), using the designated antibody (α), of lysates of HeLa cells treated with the specified siRNA, where Calnexin serves as a
30 loading control. Right: Representation of RT-sqPCR analyses of $\frac{1}{2}$ -sbsRNA1, SERPINE1 or FLJ21870 mRNA from the same lysates, where the normalized level of each transcript in the presence of Control siRNA was defined as 100. Figure 3c shows representation of RT-sqPCR analyses of FLUCSERPINE1 3'UTR or FLUC-FLJ21870 3'UTR SMD reporter

mRNA in cells that had been transiently transfected with the specified siRNA, where the normalized level of each transcript in the presence of Control siRNA was defined as 100. Figure 3d shows diagrams of expression vectors encoding $\frac{1}{2}$ -sbsRNA1(S)R, which is siRNA-resistant, $\frac{1}{2}$ -sbsRNA1(S) or FLUC with or without 12 copies of the MS2 coat protein binding site (MS2bs). Figure 3e shows western blot (upper) or RT-sqPCR (lower) before (–) or after immunoprecipitation (IP) using anti-FLAG or, as a control for nonspecific IP, mouse(m) IgG of lysates of formaldehyde-crosslinked HeLa cells that had been transiently transfected with pFLAGMS2- hMGFP and either the denoted $\frac{1}{2}$ -sbsRNA1(S) expression vector or pFLUC_MS2bs. Figure 3f shows, s in 3e, except cells were treated with Control or STAU1 siRNA. Left: Western blotting. Right: RT-sqPCR, where the co-IP efficiency indicates the level of each mRNA-derived product after IP relative to before IP. Each ratio in the presence of Control siRNA was defined as 100%. Error bars indicate s.e.m. Single asterisk, $n = 6$, $P < 0.01$; double asterisks, $n = 3$, $P < 0.05$.

10. Figure 4 shows the predicted base-pairing between the Alu element within the specified mRNA and the partially complementary Alu element within the denoted $\frac{1}{2}$ -sbsRNA. Figure 4a shows predicted base-pairing between the Alu element within the SERPINE1 or FLJ21870 3'UTR and the Alu element within $\frac{1}{2}$ -sbsRNA1, where 1 was defined as the first transcribed nucleotide of each mRNA or $\frac{1}{2}$ -sbsRNA1(S). Figures 4b, 4c, and 4d, Essentially as in 4a.

11. Figure 5 shows the mapping the 5' end of $\frac{1}{2}$ -sbsRNA1(S), and determining that the cellular abundance of $\frac{1}{2}$ -sbsRNA1(S) relative to $\frac{1}{2}$ -sbsRNA1(L) is 3:1 in HeLa cells. Figure 5a shows $\frac{1}{2}$ -sbsRNA1 mapping using an RNase Protection Assay (RPA) and three uniformly [32P]-labeled RNA probes generated *in vitro* by transcribing a subclone of Chr11 DNA (Chr11_66193000-66191383) to the designated restriction site. Figure 5b shows Single stranded DNA primer extension analysis of poly(A)+ HeLa-cell RNA (leftmost lane) or pcDNA3.1(+)/Zeo_Chrl1_66193000-66191383 in the presence of the specified dideoxy NTP (four rightmost lanes). 1 specifies the first nucleotide of $\frac{1}{2}$ -sbsRNA1(S). Figure 5c shows RTsqPCR analysis of total HeLa-cell RNA, which corroborated data shown in 5a and 5b, narrowed the 5' end of $\frac{1}{2}$ -sbsRNA1(S) to reside between positions -14 and +8, where +1 is the 5' end defined by primer extension in 5b. The analysis also demonstrated the presence of $\frac{1}{2}$ -sbsRNA1(L) at one-third the abundance of $\frac{1}{2}$ -sbsRNA1(S), i.e, at 0.25- fold the abundance of $\frac{1}{2}$ -sbsRNA1(S+L). RT was primed using random hexamers, and sqPCR was performed using one of the specified sense primers (#1-#9) and the common antisense

primer. The arrow marks the transcription start site for $\frac{1}{2}$ -sbsRNA1(S), which maps within sense primer #8 as denoted by the dotted vertical line. Figure 5d shows the sequence (SEQ ID NO: 50) of fulllength $\frac{1}{2}$ -sbsRNA1(S). Red nucleotides, Alu element; red box, putative poly(A) signal; (A)_n, poly(A) tail.

5 12. Figure 6 shows that $\frac{1}{2}$ -sbsRNA1 co-immunoprecipitates with STAU1 and is required for STAU1 binding to specific SMD targets. Figure 6a shows western blotting (upper) or RT-sqPCR (lower) of lysates of formaldehyde-crosslinked HeLa cells that had been transiently transfected with the specified siRNA and either empty vector (–) or p $\frac{1}{2}$ -sbsRNA1(S)R (+) before or after IP with anti-HA or rat IgG. After IP, each sample was
10 spiked with *in vitro* synthesized *E. coli* LACZ mRNA. The co-IP efficiency provides the level of each mRNA RT-sqPCR product after IP relative to before IP, where each ratio in the presence of Control siRNA was defined as 100%. Figure 6b shows diagrams of pFLUC-SERPINE1 3'UTR FL, which contains the full-length (FL) SERPINE1 3'UTR, and 3'UTR deletion variants. Yellow boxes, FLUC sequences; blue bars, SERPINE1 3'UTR sequences;
15 Δ; deletion; pale green boxes, 3'UTR of FLUC No SBS, which does not bind STAU1. The 5'-most pale green box ensures that ribosomes translating to the FLUC termination codon do not displace STAU1 that had been recruited to the $\frac{1}{2}$ -sbsRNA1-binding site (which is 86 nucleotides as shown in Figure 4a). Figure 6c shows western blotting (upper) and RTsqPCR (middle and lower) of lysates of HeLa-cells that had been transiently transfected with the
20 noted pFLUC-SERPINE1 3'UTR test construct and the phCMV-MUP reference plasmid. Lower: The normalized level of each FLUC mRNA in the presence of Control siRNA was defined as 100%. Error bars indicate s.e.m. Single asterisk, n = 6, *P* < 0.01; double asterisks, n = 3, *P* < 0.05.

25 13. Figure 7 shows that downregulating SERPINE1 mRNA or FLJ21870 mRNA does not decrease the efficiency with which STAU1-HA3 co-immunoprecipitates with $\frac{1}{2}$ -sbsRNA1. Essentially as in Fig. 6a, except that HeLa cells were transfected with the specified siRNA.

30 14. Figure 8 shows that RAB11FIP1 mRNA is an SMD target that is downregulated not only by STAU1 but also by $\frac{1}{2}$ -sbsRNA1. Figure 8a shows RT-sqPCR was as in Fig. 3b, except that RAB11FIP1 mRNA constituted the test mRNA. Figure 8b shows RT-sqPCR analyses of specified RNAs, where the level of SMG7 mRNA controlled for variations in RNA recovery. Figure 8c shows phase-contrast microscopy, which measured the rate of HaCaT keratinocyte wound healing after transfection with the specified siRNA for 48 hr

followed by scrape injury at 0 and 16 hr. Higher levels of SERPINE1 correlate with poorer prognoses for patients with brain tumors as well as breast, ovarian, gastric, colorectal, non-smallcell lung, renal-cell or head-and-neck cancer due to increased cancer-cell invasiveness, in at least some instances¹. In fact, SERPINE 1 stimulates “wound healing” after scrape injury to keratinocyte monolayers as monitored by the migration rate of human keratinocyte HaCaT cells into a denuded wound track². Likewise, RAB11 family interacting protein 1 (RAB11FIP1), which is also encoded by an mRNA that contains a single 3'UTR Alu element and is downregulated by STAU1 and ½-sbsRNA1 (Fig. 8a), stimulates wound-healing after scrape injury to breast epithelial MCF10A cells³. To test for ½-sbsRNA1 function in wound healing, HaCaT cells were transfected with Control siRNA, SERPINE1 siRNA, FLJ21870 siRNA, RAB11FIP1 siRNA or ½-sbsRNA1 siRNA. Each siRNA successfully downregulated its RNA target (Fig. 8b). Scrape injury repair assays that were monitored over a 16-hr period revealed that SERPINE1 siRNA or RAB11FIP1 siRNA inhibited cell movement into the wound tract, whereas ½-sbsRNA1 siRNA promoted cell movement (Fig. 8c). Thus, ½-sbsRNA1 contributes toward reducing cell migration by targeting SERPINE1 and RAB11FIP1 mRNAs for SMD. Scrape injury repair assays also indicate that FLJ21870 siRNA promoted cell movement (Fig. 8c). Since FLJ21870 function remains to be characterized, and since mechanisms that regulate FLJ21870 mRNA abundance are unknown aside from the mechanism presented here, uncovering the significance of the wound-healing result obtained using FLJ21870 siRNA requires future studies.

15. Figure 9 shows evidence that ½-sbsRNA2, ½-sbsRNA3 and ½-sbsRNA4 base-pair with particular mRNA 3'UTRs and decrease mRNA abundance, as do STAU1 and UPF1. Figure 9a shows the predicted base-pairing between the 3'UTR Alu element of CDCP1 mRNA (Acc#: NM_022842) and ½-sbsRNA2, or MTAP mRNA (Acc#: NM_002451) and ½-sbsRNA3 as well as ½-sbsRNA4, where 1 was defined as the first nucleotide listed in the NCBI data base for each mRNA or lncRNA. Figure 9b is essentially as in Fig. 3b. Error bars indicate s.e.m. Asterisk, $n = 6$, $P < 0.01$. Figure 9c shows a model for how an Alu elementcontaining ½-sbsRNA that is polyadenylated and largely cytoplasmic (red) base-pairs with a partially complementary Alu element, i.e., a half-STAU1 binding site (½-SBS), within the 3'UTR of a particular mRNA (blue) to trigger SMD. Base-pairing forms a functional SBS. The STAU1-bound SBS triggers SMD in a UPF1-dependent mechanism when translation terminates sufficiently upstream of the SBS

so that translating ribosomes do not remove bound STAU1. The ½-sbsRNA is not destroyed in the process. N, nucleus; C, cytoplasm; AUG, translation initiation codon; Ter, termination codon (which is generally, but not necessarily, a normal termination codon).

16. Figure 10 shows that SSM is Critical for Dimerization of hSTAU1⁵⁵SSM-‘RBD’5.

5 Figure 10a shows diagrams of the modular organization of domains and motifs within hSTAU1⁵⁵, its orthologs and its paralogs. Regions shown include dsRNA-binding domains (RBDs) 3 and 4 (shaded grey), which have been shown to bind dsRNA, the region that binds tubulin (TBD) in vitro (solid grey; amino acids 282-372 in STAU1⁵⁵), and the STAU-swapping motif (SSM)-‘RBD’5 region (yellow; amino acids 367-476 in hSTAU1⁵⁵, which overlaps with

10 the TBD and for which the X-ray crystal structure was reported). Figure 10B shows SSM, which typifies hSTAU1 and hSTAU2 paralogs, is conserved in all groups of vertebrates. Multiple sequence alignment was performed using vertebrate group representatives from fish (zebrafish, *Danio rerio*, NP_991124.1 (SEQ ID NO: 143)), amphibians (African clawed frog, *Xenopus laevis*, NP_001085239.1 for STAU-1 (SEQ ID NO: 144), NP_001086918.1 for

15 STAU-2 (SEQ ID NO: 145)), reptiles (Carolina anole; *Anolis carolinensis*, XP_003220668.1 (SEQ ID NO: 142)), birds (zebra finch, *Taeniopygia guttata*; XP_002188609.1 (SEQ ID NO: 141)), and mammals, i.e., human *Homo sapiens* (NP_004593.2 for STAU1-a (SEQ ID NO: 139), NP_001157856.1 for STAU2-e (SEQ ID NO: 146), STAU2-b; NP_001157853.1 (SEQ ID NO: 147)) and mouse *Mus musculus* (STAU1-

20 2; NP_001103375.1 (SEQ ID NO: 140), STAU2-2; NP_001104742.1 (SEQ ID NO: 148)). The secondary structure represented in yellow below the sequence alignments derives from the structure reported here. Figure 10C shows analytical ultracentrifugation results support a model for the existence of a SSM-‘RBD’5 monomer–dimer equilibrium in solution. The normalized apparent (*) sedimentation coefficient distribution (g(s*)) is plotted as a function of apparent

25 Svedbergs (s*). Figure 10D shows ‘RBD’5 homodimerization in human cells requires SSM. HEK293T cells (1 x 10⁷/150-mm dish) were transiently transfected with a pEGFP-‘RBD’5 (5 µg) and either pmRFP-SSM-‘RBD’5 (5 µg) or pmRFP-‘RBD’5 (5 µg). Alternatively, cells were transfected with pEGFP-SSM-‘RBD’5 (5 µg) and either pmRFP-SSM-‘RBD’5 (5 µg) or pmRFP-‘RBD’5 (5 µg). Cell lysates were immunoprecipitated in the presence of RNase A

30 using anti(α)-GFP or, to control for nonspecific immunoprecipitation (IP), mouse(m)IgG. Western blotting (WB) using the specified antibody, where calnexin serves to control for IP specificity. Results are representative of three independently performed experiments.

17. Figure 11 shows that some but not All InvertebrateSTAU Proteins have an SSM. Multiple protein sequence alignments of the STAU proteins in Figure 10B and STAU proteins from the following invertebrates: acorn worm, *Saccoglossus kowalevskii*, XP_002731114.1 (SEQ ID NO: 149); deer tick, *Ixodes scapularis*, XP_002433902.1 (SEQ ID NO: 150); water flea, *Daphnia pulex*, EFX74549.1 (SEQ ID NO: 151); fruit fly, *Drosophila melanogaster*, NP_476751.1 (SEQ ID NO: 152); mosquito, *Anopheles gambiae*, XP_308394.4 (SEQ ID NO: 153); roundworm, *Caenorhabditis elegans*, CCD62871.1 (SEQ ID NO: 154); red flour beetle, *Tribolium castaneum*, EFA11564.1 (SEQ ID NO: 155). The dotted lines between SSM and 'RBD'5 in this figure do not exist in Figure 1B because weak similarities between *D. melanogaster* STAU-A, which lacks an SSM, and the SSM of other proteins shifts SSM sequences to the left.

18. Figure 12 shows the structure of the SSM-RBD5 Domain-Swapped Dimer. Figure 12A shows a cartoon of the secondary structure of SSM-'RBD'5 where one molecule of SSM-'RBD'5 is green, the other molecule is blue. The two α -helices of SSM contact the two α -helices of 'RBD'5. Dotted lines represent the assumed linkage between SSM and 'RBD'5 since the crystal structure lacks connecting density. The rightmost dimer structure is rotated 90° around the X-axis relative to the leftmost dimer structure. Figure 12B shows a close-up of the interaction between the SSM α -helices (green $\alpha 1$ and $\alpha 2$) and the 'RBD'5 α -helices (blue $\alpha 1$ and $\alpha 2$). Important residues are shown as stick representations, colored yellow, and labeled. The hydrogen-bonding interaction is shown as a dotted line.

19. Figure 13 shows support for SSM-'RBD'5-SSM-'RBD'5 Domain-Swapping, i.e., a trans arrangement. Figure 13A shows possible cis and trans arrangements of symmetry mates in the structure (left) and PISA results (right). Figure 13B shows interactions of the unfavored, i.e., cis orientation. Residues of this crystal contact that were used to design SSM-'RBD'5 (E424H, D427V) are shown.

20. Figure 14 shows a comparison of the Degenerate 'RBD'5 with a RBD that Binds dsRNA. Figure 14A shows the structure-based sequence alignment of hSTAU1 'RBD'5 (top green sequence) (SEQ ID NO: 156) and hTRBP2 RBD1 (bottom grey sequence) (SEQ ID NO: 157). Conserved hydrophobic amino acids are shaded yellow, and conserved Tyr and Lys residues are shaded cyan and green, respectively. Key residues discussed in the text are blue if positively charged and red if negatively charged. Small upward-pointing arrowheads indicate the position of residues reported for hTRBD2 RBD1 and RBD2 that interact directly with dsRNA. Figure 14B shows a cartoon of superposed hSTAU1 'RBD'5 (green) and hTRBP2

RBD1 (grey) produced by Dali. Figure 14C shows X-ray crystal structure of hTRBP2 RBD2 in complex with dsRNA. hTRBP2 RBD2 is shown rather than hTRBP2 RBD1, which is more similar to hSTAU1 'RBD'5, since hTRBP1RBD1 lacks a complete Loop 2 (L2). Protein is blue and in cartoon-form, and dsRNA is in stick-representation. The three major interacting RBD
5 regions of typical RBDs are approximated using round-edged enclosures to illustrate the important secondary structures. Figure 14D shows a cartoon of either hTRBP2 RBD1 or STAU1 'RBD'5 superimposed on hTRBP2 RBD2, shown in relation to the position of dsRNA in the TRBP2 RBD2 structure. Vacuum electrostatic potentials were generated using PyMOL to illustrate charge variance, where blue is positive, red is negative, and white is neutral on the
10 surface representation of each protein. As in C, regions necessary to interact with dsRNA are indicated.

21. Figure 15 shows a structural comparison of STAU1 RBD3 (hSTAU1 RBD3 SEQ ID NO: 158; dSTAUa RBD3 (SEQ ID NO: 159)) and STAU1 'RBD'5 (SEQ ID NO: 160) Through the Highly Similar NMR Structure of *D. melanogaster* STAU RBD3. Figure 15A
15 shows structure based sequence alignment between proteins. Figure 15B shows vacuum electrostatic potentials were calculated using PyMOL for RBD3 (surface) and are shown in complex with the bound hairpin RNA (sticks). The hSTAU1 'RBD'5 electrostatic model from Figure 14D is shown for comparison.

IV. DETAILED DESCRIPTION

22. The present invention may be understood more readily by reference to the following detailed description of preferred embodiments of the invention and the Examples included therein and to the Figures and their previous and following description.

23. Before the present compounds, compositions, articles, devices, and/or methods
25 are disclosed and described, it is to be understood that this invention is not limited to specific synthetic methods, specific recombinant biotechnology methods unless otherwise specified, or to particular reagents unless otherwise specified, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

30 24. As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a pharmaceutical carrier" includes mixtures of two or more such carriers, and the like.

25. Ranges may be expressed herein as from “about” one particular value, and/or to “about” another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent “about,” it will be understood that the particular value forms another embodiment. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint.

26. In this specification and in the claims which follow, reference will be made to a number of terms which shall be defined to have the following meanings:

27. “Optional” or “optionally” means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not.

28. “Primers” are a subset of probes which are capable of supporting some type of enzymatic manipulation and which can hybridize with a target nucleic acid such that the enzymatic manipulation can occur. A primer can be made from any combination of nucleotides or nucleotide derivatives or analogs available in the art which do not interfere with the enzymatic manipulation.

29. “Probes” are molecules capable of interacting with a target nucleic acid, typically in a sequence specific manner, for example through hybridization. The hybridization of nucleic acids is well understood in the art and discussed herein. Typically a probe can be made from any combination of nucleotides or nucleotide derivatives or analogs available in the art.

30. As used herein, “chemical” refers to any reagent that can bind, mutate, dissolve, cleave, initiate a reaction that results in a new agent, or alter the confirmation of a target agent. An example of a chemical would be a small molecule.

31. As used herein, “pioneer round of translation” or “pioneering round” refers to an initial round of mRNA translation prior to steady-state translation. The round is characterized by the presence of CBP80 and CBP20 as the CAP binding proteins, PABP2 as a polyadenylation binding protein, and involves Upf2 and Upf3 or Upf3X.

32. In one aspect disclosed herein are methods of screening, methods of treatment, and compositions related to Staufen 1 mediated mRNA decay (SMD), targets of SMD and diseases associated with truncated or erroneous proteins.

Staufen 1

33. Mammalian Staufen1 (Stau1) is an RNA binding protein that binds to extensive RNA secondary structures, primarily through one or more double-stranded RNA-binding domains. In mammals, the Stau1 gene is ubiquitously expressed and generates protein isoforms having apparent molecular weights of 55 and 63 kDa. The 55-kDa isoform
5 associates with 40S and 60S ribosomal subunits and co-localizes with the rough endoplasmic reticulum. A role for Stau1 in mRNA transport and translational control has been inferred from its presence in RNA granules that migrate within the dendrites of hippocampal neurons in a microtubule-dependent manner, as well as its encapsidation together with HIV-1 RNA in virus particles. Additionally, Stau1 interacts with telomerase
10 RNA, suggesting that it functions during DNA replication, cell division or both, possibly by influencing telomerase RNA processing or RNP assembly or localization.

Stau1 mediated mRNA decay (SMD)

34. Staufen1 (STAU1)-mediated mRNA decay (SMD) degrades translationally active mRNAs that bind the double-stranded (ds)RNA binding protein STAU1 within their
15 3'-untranslated regions (3'UTRs). The STAU1 binding site (SBS) is within ADP ribosylation factor 1 (ARF1) mRNA as a 19-basepair stem with a 100-nucleotide apex. However, comparable structures within the 3'UTRs of other SMD targets were not identified. Herein is disclosed that SBSs can be formed in *cis* by intramolecular base-pairing within an mRNA 3'UTR, or in *trans* by imperfect base-pairing between an Alu
20 element within the 3'UTR of an SMD target and another Alu element within a cytoplasmic and polyadenylated long noncoding RNA (lncRNA). When translation terminates sufficiently upstream of an SBS, association of the ATP-dependent RNA helicase UPF1 with SBS-bound STAU1 triggers mRNA decay analogously to how UPF1 association with an exon-junction complex that resides sufficiently downstream of premature termination
25 codon triggers nonsense-mediated mRNA decay. Individual lncRNAs can downregulate a subset of SMD targets, and distinct lncRNAs can downregulate the same SMD target. These are previously unappreciated functions for ncRNAs and Alu elements.

35. Stau1 is involved in mRNA decay. Also, Stau1-mediated mRNA decay involves the nonsense-mediated mRNA decay (NMD) factor Upf1. In mammalian cells, the
30 expression of protein-encoding genes requires a series of steps in which pre-mRNA is processed to mRNA in the nucleus before mRNA is translated into protein in the cytoplasm. These steps are subject to quality control to ensure that only completely processed mRNA is exported to the cytoplasm. One form of quality control, called mRNA surveillance or

NMD. NMD in mammalian cells is generally a splicing-dependent mechanism that degrades newly synthesized mRNAs that prematurely terminate translation more than 50-55 nucleotides upstream of an exon-exon junction as a means to prevent the synthesis of potentially harmful truncated proteins. By so doing, NMD precludes the synthesis of the encoded truncated proteins, which can function in deleterious ways. NMD also targets naturally occurring mRNAs such as certain selenoprotein mRNAs and an estimated one-third of alternatively spliced mRNAs, some of which encode functional protein isoforms.

36. The dependence of NMD on splicing reflects the deposition of an exon junction complex (EJC) of proteins ~20-24 nucleotides upstream of splicing-generated exon-exon junctions. This EJC includes NMD factors Upf3 (also called Upf3a) or Upf3X (also called Upf3b), Upf2 and, presumably, Upf1. Upf3 and Upf3X appear to play a comparable role in NMD, although different isoforms of Upf3 can form distinct protein complexes. Other constituents of the EJC include Y14, RNPS1, SRm160, REF/Aly, UAP56, Mago, Pinin, and eIF4AIII. EJCs are present on mRNA that is bound at the cap by the mostly nuclear cap binding protein (CBP)80-CBP20 heterodimer, which is consistent with data indicating that NMD targets CBP80-bound mRNA during a pioneer round of translation.

37. Mammalian Stau1 binds the NMD factor Upf1 and the 3' untranslated region (UTR) of mRNA that encodes ADP-ribosylation factor (Arf)1. As a consequence, Stau1 mediates Arf1 mRNA decay in a mechanism that differs from NMD by occurring independently of splicing or Upf2 and Upf3X. Analogously to the Stau1-mediated mRNA decay (SMD) of Arf1 mRNA, artificially tethering Stau1 downstream of a normal termination codon also reduces mRNA abundance in a mechanism that depends on the normal termination codon and Upf1 but neither splicing nor Upf2, Upf3 or Upf3X. Notably, Stau1 plays no detectable role in the EJC-dependent NMD of either β -globin or glutathione peroxidase 1 mRNA.

ALU elements

38. Disclosed herein, Stau1 can also bind to a binding site created by the imperfect base pairing on an ALU element in the 3' UTR of the mRNA of a transcript and an ALU element contained within long noncoding RNA. This is the first description of a function for long noncoding RNA (lncRNA). Whether present in the lncRNA or in the 3' UTR of the mRNA of a transcript, an ALU element is typically an approximately 300 base pair nucleic acid construct classified as short interspersed elements. ALU elements can vary in

sequence, but generally correspond to the consensus sequence as set forth in SEQ ID NO: 3.

Staufen1-Mediated mRNA Decay by Swapping a Conserved Motif and a Degenerate Double-Stranded RNA-Binding Domain

5 39. Human (h)STAU1 has 496- and 577-amino acid isoforms (NCBI Gene ID:6780; hSTAU1⁵⁵ and hSTAU1⁶³, respectively), each of which contains RBDs 2-5, and an additional isoform with six amino acids inserted into hSTAU1⁵⁵RBD3 that in the highly similar mouse ortholog diminishes dsRNA binding. Only RBD3 and RBD4 bind dsRNA in mammalian cells (thus, hereafter RBD2 and RBD5 are referred to as, respectively, 'RBD'2 and 'RBD'5), and
10 RBD3 binds dsRNA with higher affinity than does RBD4. All three hSTAU1 isoforms also contain a tubulin-binding domain (TBD) situated between RBD4 and 'RBD'5 that probably binds tubulin based on in vitro studies of the highly similar mouse STAU1 TBD.

40. The hSTAU1 paralog, hSTAU2, has 479-, 504-, 538- and 570-amino acid isoforms (NCBI Gene ID: 27067; hSTAU2⁵², hSTAU2⁵⁶, hSTAU2⁵⁹, and hSTAU2⁶², respectively), each
15 of which contain RBDs 2, 3 and 4, and only a partial N-terminal region of what would be hSTAU1 'RBD'5; additionally, hSTAU2⁵⁶ and hSTAU2⁶² have a complete RBD1, whereas hSTAU2⁵² and hSTAU2⁵⁹ contain a truncated RBD1. hSTAU2 isoforms also have a putative TBD that shares 17% identity with the hSTAU1 TBD but has yet to be shown to bind tubulin. Like hSTAU1, hSTAU2 mediates not only mRNA decay but also mRNA localization. Each
20 paralog and even some of their isoforms function and localize differently within cells.

41. The X-ray crystal structure of a region of hSTAU1 includes a new motif that called the STAU-swapping motif (SSM). SSM is conserved in all examined vertebrate STAU homologs, resides N-terminal to 'RBD'5, to which it is connected by a flexible linker, and is responsible for forming hSTAU1 dimers both in vitro and in vivo. The two α -helices of SSM
25 form a domain-swapped interaction with the N-terminal α -helix of 'RBD'5 so as to result in full-length hSTAU1 dimerization. This is a previously unappreciated role for an RBD that no longer binds dsRNA. In cells, disrupting hSTAU1 dimerization either by deleting SSM- 'RBD5' from one of the two interacting proteins, or by expressing exogenous 'RBD'5 in the presence of two full-length proteins, reduces the ability of full-length hSTAU1 to co-
30 immunoprecipitate with hUPF1, and thus, reduces the efficiency of SMD.

42. Herein is shown new motif that typifies STAU homologs from all vertebrate classes and is responsible for human (h)STAU1 homodimerization. The crystal structure reveals that this motif, now named SSM for 'Staufen-swapping motif', and dsRNA-binding domain

(RBD)5, which has diverged from a prototypic RBD and no longer binds dsRNA, mediate domain swapping: the two SSM α -helices of one molecule interact mainly through a hydrophobic patch with the two 'RBD'5 α -helices of a second molecule, and vice versa. 'RBD'5 adopts the canonical α - β - β - α fold of a functional RBD, but lacks residues and key features needed to bind duplexed RNA. In cultured cells, the SSM and N-terminal α -helix of 'RBD'5 are sufficient for hSTAU1-hSTAU1 dimerization, and dimerization is critical for efficient SMD.

Methods of identifying SMD targets

43. Expression of truncated or erroneous proteins can have severe effects on a person expressing said proteins. The truncated or erroneous proteins can have dominant negative effects or result in gain-of function mutations that can have severe consequences and result in cancers and other disorders. It is the role of SMD to reduce the occurrence of the truncated or erroneous proteins before they are expressed. Significantly, knowing the targets of SMD would provide new targets for treatments of disease, assessment of risks groups for disease, and diagnosis of a disease. Accordingly, disclosed herein are methods of identifying a Stau1 (Stau1)-binding site (SBS) that mediates Stau1-mediated mRNA decay (SMD) comprising the steps of a) down-regulating Stau1 or an ALU element-containing long noncoding RNA (lncRNA); b) identifying transcripts that are up-regulated at least 1.8-fold upon downregulation of Stau1 or the ALU element-containing lncRNA, wherein transcripts that are up-regulated at least 1.8-fold are SMD targets; and c) identifying an ALU element within the 3' UTR of the transcript of the SMD target, wherein the ALU element comprises a first strand of the SBS.

44. The methods disclosed herein utilize the down-regulation of Stau1 or lncRNA to identify SMD targets as such targets will increase in amount or expression by virtue of not being degraded by SMD. It is also understood that one method of down regulating Stau1 or lncRNA is through the use of siRNA. Further it is understood and discussed herein that there are many methods that can be used to detect the up-regulation of genes, mRNA, or proteins that are known in the art and include but are not limited to microarray, northern blot, southern blot, and western blot.

45. There are many SMD binding sites (SBS) and the sequence and identity of said sites is associated with SMD targets identified in the Examples below. Thus, disclosed herein are methods of identifying SBS, wherein the SMD target is selected from the group consisting of IFI44, FBLIM1, CLDN11, PDLIM3, OASL, TAF7L, NOX4, AIM1,

SERPINE1, DCP2, EIF5A2, CEP135, LRRFIP1, ZFP90, TP53, PHLPP2, EHHADH, CCDC125, NUA2, AKT2, APPL1, COL16A1, RIPK1, MGC14799, GNAS, DFFA, CSDA, SEC61A1, PSMD12, , C4orf9, FLJ10613, GNE, FLJ30656, LOC149603, TEGT, MCM4, LOC63929, KIAA0186, PRKAR2A, NUTF2, GDF1, PAICS, TMPO, AAMP, IF, 5 FN1, IFIT2, SCG2, STMN3, GAP43, CKMT1, OSF-2, FLJ25348, GNG4, FLJ33505, FBLP-1, IGFBP5, ITGB3, IFIT4, ALP, TAGLN, TFPI2, CTNNAL1, THBS1, PTPRO, CEB1, MGC29643, FLJ20035, AIM1, C14orf141, DUSP6, G1P2, FLJ20637, SLAMF1, SCLY, IL7R, ARHGDIB, ACTA2, CXCL1, RIG-1, IFIT1, FLY34064, MGC19764, TLE4, DACT1, C9orf39, JUN, IGA4, TNC, FLJ13621, MICAL2, TMSNB, PTGER4, C14orf128, 10 GDAP1, ZFP36L1, UNG, CDKAL1, ALDH1A3, PSMB9, PHF11, BANK1, KIAA0143, GBP1, EPPK1, ACTN1, RNF20, LRRFIP1, LOC150759, DPYSL3, PSK-1, ZFP90, TP53, LOC148418, TGFB1I1, ETV1, ARHGAP19, SOCS2, CDK5R1, SDS3, SCDGF-B, HDAC8, HMGB3, KIAA0931, DFKZp761K1423, PTPRF, TXNRD3, CGI-72, MGC15634, HES1, C11orf9, and TOIA1.

15 46. It is further understood that because the SBS can be the result of imperfect base pairing between an ALU element contained within lncRNA and ALU element on an SMD target; where the screening method comprises the down regulation of Stau1, the method further comprises step d. identifying an ALU element in a cytoplasmic polyadenated lncRNA that contains an ALU element that base pairs with the ALU element in the SMD 20 target, wherein the ALU element of the lncRNA comprises a second strand of the SBS. It is understood and disclosed herein that there are many methods that may be used to uncover the identity of an lncRNA including, but not limited to, co-immunoprecipitation and the computation analyses employed herein. Thus, for example disclosed herein are methods of identifying a SBS of SMD comprising a) transiently expressing an ALU element-containing 25 lncRNA linked to a reporter gene; and b) immunoprecipitating the ALU element-containing lncRNA using an anti-reporter protein antibody; wherein ALU elements within RNA that encode proteins other than Stau1 that are co-immunoprecipitated with the ALU element of the lncRNA is a target SMD binding site (see, for example, Example 6).

Methods of screening

30 47. An agent that is able to modulate SMD is useful for the treatment and study of SMD related disorders. Thus, specifically disclosed are methods of screening for an agent that modulates Stau1-mediated mRNA decay (SMD) comprising incubating the agent with a stably transfected cell comprising a reporter gene with a 3' UTR ALU element; a lncRNA

containing a corresponding ALU element; and Stau1, and assaying the amount of mRNA of the reporter gene in the cell. An increase or decrease in the amount of mRNA of the reporter gene relative to the amount of mRNA of the reporter gene in the absence of the agent indicates an agent or substance that modulates SMD activity. Also disclosed are methods of identifying an agent that binds a SBS comprising contacting the SBS with the agent to be screened, wherein the SBS comprises an ALU element from a SMD target and an ALU element from a lncRNA that base pairs with the ALU element from the SMD target.

48. It is understood that another means by which one of skill in the art may use to screen for agents that modulated SMD is through the detection or measurement of complexes such as an SMD-target-Stau1 complex. It is understood that by "SMD complex" is any combination of one or more of the essential components of SMD. Thus, for example, specifically disclosed are screening methods wherein the complex comprises Upfl and Stau1. Also disclosed are screening methods wherein the complex comprises one of Upfl and Stau1. Thus, for example disclosed are methods of screening for an agent that modulates SMD comprising a) incubating the agent with an SMD target and Stau1 forming an agent- SMD target-Stau1 mixture, and b) assaying the amount of SMD target-Stau1 complex present in the mixture, wherein a increase or decrease in the amount of SMD target-Stau1 complex relative to the amount of SMD target -Stau1 complex in the absence of the agent indicates that the agent promotes or inhibits SMD, respectively. Also disclosed are methods of screening for an agent that modulates SMD comprising a) incubating the agent with an SMD target and Stau1 forming an agent- SMD target-Stau1 mixture, and b) assaying the amount of SMD target present in the mixture, wherein a increase or decrease in the amount of SMD target relative to the amount of SMD target in the absence of the agent indicates that the agent inhibits or promotes SMD, respectively.

49. Also disclosed is a method of screening for an agent that modulates Stau1-mediated mRNA decay (SMD) comprising incubating the agent to be screened with a stably transfected cell comprising a reporter gene with a nonsense-mutation and Stau1, and assaying the amount of SMD in the cell, wherein a increase or decrease in the amount of mRNA relative to the amount of mRNA in the absence of the agent indicates a agent that modulates SMD activity.

50. It is understood that many fragments or minor variants of the disclosed protein can be used in the disclosed methods. Thus, specifically disclosed are screening methods

wherein the Stau1 has at least 80%, 85%, 90%, or 95% identity to the sequence set forth in SEQ ID NO: 2, or an SMD-active fragment thereof. It is also understood that the disclosed methods contemplate that the SBS within the SMD target comprises an ALU element in the 3' UTR of the SMD target. It is understood that ALU sequences can vary from SMD target to target but typically select the ALU element consensus sequence set forth in SEQ ID NO: 3. Therefore disclosed herein are methods wherein the ALU element has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% identity to the consensus ALU element set forth in SEQ ID NO: 3.

51. Disclosed herein are methods of screening for an agent that modulates Stau1-mediated mRNA decay (SMD) comprising, administering an agent to a system, wherein the system comprises the components for SMD activity, and assaying the effect of the agent on the amount of SMD activity in the system, a change in the amount of SMD activity present in the system compared to the amount of SMD activity in the system in the absence of the agent indicates the agent is a modulator. Thus, specifically contemplated and herein disclosed are methods of modulating Stau1-mediated mRNA decay (SMD) activity comprising administering an agent, wherein the agent is identified by the disclosed screening methods.

52. The agents that can be used to modulate SMD can function by inhibiting the binding of Stau1 to its binding site. For example, an agent that competitively binds a Stau1 binding site can inhibit SMD. Therefore, specifically disclosed are methods of identifying an agent that binds a Stau1 binding site comprising contacting the agent to be screened with the Stau1 binding site. Also disclosed are methods of identifying an agent that binds a Stau1 binding site wherein one strand of the Stau1 binding has at least 80%, 85%, 90%, or 95% identity to the sequence set forth in SEQ ID NO: 3.

53. Also disclosed are screening and treatment methods, wherein the agent binds Upf1, an ALU element containing lncRNA, an SMD target, the SBS of an SMD target (such as for example an ALU element in the 3' UTR of the SMD target), or Stau1. As used herein, "binds" or "interacts" means to affect an agent either directly or indirectly through cooperative function, competitive inhibition, non-competitive inhibition, binding, or contacting the agent, a target molecule, an accessory molecule, or alternative portion of a system so as to effect at least one function. The interaction can be stimulatory or cooperative in nature having an additive or synergistic effect. The interaction can also result in the inhibition of a process or target molecule.

54. The disclosed methods function by modulating SMD. Herein, it is understood that by “modulation” or “modulating” is meant either an increase or a decrease in SMD activity. Alternatively, Staufen 1 can modulate mRNA transcripts through the stabilization or destabilization of an mRNA. Whether SMD levels are increased or decreased in a
5 subject with a condition resulting from a mutation that generates a nonsense codon (including but not limited to a nonsense mutation) depends on the particular mRNA that is affected, the binding of Staufen1 and, where binding of Stau1 occurs. A decrease in SMD activity could increase the amount of mRNA that is available for translation. Thus, for example, modulation can be measured by, e.g., comparing the level of the mRNA harboring
10 a premature or alternative termination codon relative to an unaffected cellular RNA to the level of that mRNA lacking a premature or alternative termination codon to the same unaffected cellular RNA. Subsequent nonsense suppression could be used to increase the amount of the encoded full-length protein. Conversely, an increase in SMD activity would decrease the amount of mRNA that is available for translation so as to reduce production of
15 the encoded truncated protein. Thus, it is understood and herein contemplated that the disclosed methods can be used to modulate the expression of genes by modulating SMD (e.g., to decrease mRNA from a truncated protein, one would increase SMD activity. A decrease in SMD activity would be used to increase the amount of mRNA of a truncated protein available). Notably, SMD can also target mRNAs that do not have a premature or
20 alternative termination codon, providing another means by which gene expression could be regulated.

55. Thus, for example, specifically disclosed are methods wherein the modulation is a decrease in Stau1-mediated mRNA decay. A “decrease” can refer to any change that results in a smaller amount of Stau1 mRNA mediated decay. Thus, a “decrease” can refer
25 to a reduction in an activity. An agent is also understood to decrease the genetic output of a gene when the genetic output of the gene product with the agent is less relative to the output of the gene product without the agent. Also for example, a decrease can be a change in the symptoms of a disorder such that the symptoms are less than previously observed. In the case of a decrease in Stau1 mRNA mediated decay, it is understood and herein
30 contemplated that a decrease can include, but is not limited to, a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% reduction. A decrease can but does not have to result in the complete ablation of a substance or activity. Therefore, for example, a decrease in SMD would result in the presence of more gene products with early or alternative

termination sites (i.e., a decrease in SMD activity). It is understood that the term “inhibits” or “inhibition” refers to any degree of decrease as compared to a control. Thus, for example, “inhibition” can refer to a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% reduction as compared to a control.

5 56. Also disclosed are methods wherein the modulation is an increase in Stau1-mediated mRNA decay. An “increase” can refer to any change that results in a larger amount of a Stau1 mediated mRNA decay activity. Thus, for example, an increase in the amount in SMD of a particular mRNA can include but is not limited to a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% increase. It is understood and herein
10 contemplated that an increase in SMD would result in a subsequent decrease in the amount of gene products with early or alternative termination sites.

 57. It is understood and herein contemplated that the disclosed screening and treatment methods can utilize the modulation of the level of mRNA or protein expression. It is also understood and herein contemplated that Stau1 can increase or decrease the
15 abundance of an mRNA independently of SMD. Likewise, it is understood and herein contemplated that not all SMD activity occurs through the involvement of Stau1 and Upf1, but can be Upf1 independent. By “modulating the level of mRNA” is meant that the abundance of a target transcript can be increased or decreased by the expression of Stau1. The ability to modulate the abundance of target genes can be achieved in conjunction with
20 or independently of Upf1. It is understood that such effect can be either direct or indirect. By “direct effect” is meant that Stau1 acts on the targeted mRNA itself and by “indirect effect” is meant that Stau1 acts by an intermediary, including for example, a nucleic acid. Thus, Stau1 down-regulation can result either directly or indirectly in the down regulation of target mRNA levels. It is understood that whether the effect is direct or indirect will depend
25 on the target gene. One of skill in the art will be able to determine whether the effect is direct or indirect given the target gene. Thus specifically disclosed and herein contemplated are methods of identifying genes modulated by the down-regulation of Stau1 or an ALU containing lncRNA comprising incubating a agent that down-regulates SMD with a stably transfected cell comprising Stau1 and one or more selected genes comprising one or more
30 nonsense-mutations, and assaying the amount of protein expressed of the gene being screened or mRNA present for the gene being screened, wherein a increase or decrease in the amount of protein or mRNA relative to the amount of protein or mRNA in the absence of the siRNA indicates a gene that is modulated by Stau1 activity.

58. It is understood that these methods can be used to identify genes that are up-regulated or down regulated by the down-regulation of Stau1 as well as identify those genes whose abundance increases or decreases with the down-regulation of Stau1. It is understood and herein contemplated that one example of an agent that can down regulate
5 SMD is small interfering RNA (siRNA) to a component of SMD such as Stau1 or lncRNA.

59. Although Stau1 can modulate mRNA levels independently of Upf1 and SMD, dependent modulation can also occur. Thus, disclosed herein are methods of identifying genes modulated by the down-regulation of Upf1 comprising a) transfecting a small
10 interfering RNA (siRNA) that down-regulates SMD into a cell comprising Upf1 and one or more selected genes comprising one or more nonsense-mutations, and b) assaying the amount of protein expressed of the gene being screened or mRNA present for the gene being screened, wherein a increase or decrease in the amount of protein or mRNA relative to the amount of protein or mRNA in the absence of the siRNA indicates a gene that is
15 modulated by Upf1 activity. Also disclosed are method of identifying genes modulated by the down-regulation of SMD comprising a) transfecting a small interfering RNA (siRNA) that down-regulates SMD into a cell comprising UPf1, Stau1, and one or more selected genes comprising one or more nonsense-mutations, and b) assaying the amount of protein expressed of the gene being screened or mRNA present for the gene being screened,
20 wherein a increase or decrease in the amount of protein or mRNA relative to the amount of protein or mRNA in the absence of the siRNA indicates a gene that is modulated by SMD activity. It is understood that the siRNA can be, for example, Upf1 siRNA or Stau1 siRNA.

60. Various assays are known in the art that can be used to measure mRNA levels or protein expression. For example, mRNA levels can be measured by microarray or RT-PCR.
25 Protein expression can be measured by Western blot. It is understood and herein contemplated that the disclosed methods of identifying genes can be used with any method of measuring protein expression or mRNA levels known to those of skill in the art.

61. Also disclosed are methods of modulating the level of an mRNA comprising administering to a subject an effective amount of an agent that modulates Stau1, wherein
30 the modulation Stau1 directly or indirectly modulates the mRNA. Also disclosed are methods of treating a disorder in a subject comprising administering to the subject an agent, wherein the agent modulates Stau1 wherein the modulation of Stau1 modulates that level of mRNA abundance of another gene.

62. The disclosed compositions and methods can be used to evaluate the expression of genes involved in SMD and in particular in or as a result of the pioneer round of translation. Specifically contemplated are methods wherein mRNA from a system comprising a nonsense-mutation is assayed using a micro array. Genes identified as having
5 significantly (as determined by the manufacturers specifications of the array) increased or decreased expression are comodulators of SMD.

63. Disclosed are chips where at least one address is the sequences or part of the sequences set forth in any of the nucleic acid sequences disclosed herein. Also disclosed are chips where at least one address is the sequences or portion of sequences set forth in any
10 of the peptide sequences disclosed herein.

64. Also disclosed are chips where at least one address is a variant of the sequences or part of the sequences set forth in any of the nucleic acid sequences disclosed herein. Also disclosed are chips where at least one address is a variant of the sequences or portion of sequences set forth in any of the peptide sequences disclosed herein.

15 **Methods of treating**

65. The invention described herein in one aspect relates to methods of treating a disorder in a subject comprising administering to the subject an agent, wherein the agent modulates Stau1-mediated mRNA decay (SMD). For example disclosed herein are methods of treating a disorder associated with expression of a truncated or erroneous
20 protein in a subject comprising administering to the subject an agent, wherein the agent increases SMD of the gene encoding the target protein in the subject.

66. It is understood and herein contemplated that the term “treating” can refer to any method that improves a disorder in a subject. The improvement can include but is not limited to a decrease in one or more symptoms of the disorder such that the disorder is
25 reduced. Treating is understood to include small improvements in the disorder up to and including the complete ablation of the disorder. For example, the treatment can result in a 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% reduction in the disorder.

67. It is understood and herein contemplated that the disorders to be treated can be the result of or related to the expression truncated or erroneous proteins. Such proteins can
30 be the result of aberrant mRNA splicing that leads to premature termination of the mRNA. It is understood that the mRNA that encodes the truncated or erroneous protein can comprise at least one ALU element in its 3' UTR. There are many disorders that can be treated with the disclosed methods. It is understood and herein contemplated that a

“disorder” means any inherited or acquired disease or condition associated with Stau1 mediated mRNA decay that has a negative effect relative to the wild-type state. For example, the disorder can be inherited genetic disorder. Inherited genetic disorders are disorders that result from the presence of an abherent gene or genes that alter the way an interaction, system or pathway works. As used herein, “system” refers to any cell, organism, or in vitro assay or culture. Such a system includes components necessary for SMD activity. Such components can include, for example, but are not limited to Stau1 and Upf1. Thus for example, specifically contemplated are methods disclosed herein wherein the disorder is a genetic disorder, and wherein the genetic disorder can be selected from the group consisting of cystic fibrosis, hemophilia, mucopolysaccharidoses, muscular dystrophy, anemia, glycolytic enzyme deficiency, connective tissue disorder, DNA repair disorder, dementia, Diabetes mellitus type II, Alzheimer’s disease, Marfan’s syndrome, β -Thalasimia, Neurofibromatosis, Hypercholesterolemia, Sandhoff disease, epidermolysis bullosa simplex, insulin resistance, maple syrup urine disease, hereditary fructose intolerance, inherited immunodeficiency, inherited cancer, carbohydrate metabolism disorder, amino acid metabolism disorder, lipoprotein metabolism disorder, lipid metabolism disorder, lysosomal enzymes disorder, steroid metabolism disorder, purine metabolism disorder, pyrimidine metabolism disorder, metal metabolism disorder, porphyrin metabolism disorder, and heme metabolism disorder.

68. Also for example, a disorder can be dementia. Dementias can include but are not limited to Alzheimer’s, Lewy Body dementia, Binswanger’s dementia, or dementias associated with Parkinson’s Disease, progressive supranuclear palsy, Huntington’s disease, Pick’s disease, Creutzfeldt-Jakob disease, Gerstmann-Straussler-Scheinker disease, AIDS, or trauma.

69. Disorders can also include acquired disorders. Acquired disorders are disorders that result from some external insult or injury, or from an unknown mechanism that is not derived from a genetic characteristic. Thus by “acquired disorder” is meant any disorder that lacks a clear genetically inherited link. For example, an infection or malignancy without a clear genetically inherited link. For example, acquired disorders can result from chemical exposure, radiation exposure, or random mutation in a gene that was not present in the subject earlier. Thus for example, an acquired disorder can comprise a cancer.

70. Also disclosed are methods of the invention, wherein the disorder is an acquired disorder. The acquired disorder is, by way of example but not by way of a limitation, a cancer. Such a cancer may be related to mutations such as mutations in p53 or BRCA-1.

71. The disclosed compositions can be used to treat any disease where uncontrolled
5 cellular proliferation occurs such as cancers. A representative but non-limiting list of cancers that the disclosed compositions can be used to treat is the following: lymphoma, B cell lymphoma, T cell lymphoma, leukemia, carcinoma, sarcoma, glioma, blastoma, neuroblastoma, Ewing's sarcoma, gastric cancer, lung cancer, plasmacytoma, histiocytoma, melanoma, mycosis fungoide, hypoxic tumor, myeloma, metastatic cancer, bladder cancer,
10 brain cancer, nervous system cancer, head and neck cancer, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, liver cancer, colon cancer, cervical cancer, breast cancer, epithelial cancer, renal cancer, genitourinary cancer, pulmonary cancer, esophageal carcinoma, hematopoietic cancers, testicular cancer, and colorectal cancer.

72. Disclosed and herein contemplated are methods wherein the SMD occurs in or
15 as a result of the pioneering round of translation. Notably, SMD can also occur during steady-state translation. During steady-state translation, can target eIF4E-bound mRNA.

73. As used herein, "subject" refers to any cell, tissue, system, or organism used to study or treat a disorder relating to SMD, including, for example, human patients with conditions that result from SMD or cell lines used to study aspects of SMD. Thus, in one
20 embodiment the subject is a mammal. It is specifically contemplated that mammal can include but is not limited to human or non-human primate.

74. Also disclosed are methods of treating a disorder in a subject comprising administering to the subject a substance, wherein the substance modulates Stau1-mediated mRNA decay. Modulation of Stau1-mediated mRNA decay can result in a decrease in
25 SMD. The decrease in SMD would increase the abundance of the nRNA containing a premature or alternative termination codon.

75. Specifically disclosed herein are methods of facilitating Stau1-mediated mRNA decay comprising contacting a system comprising the components for SMD with Stau1. Also disclosed are methods of facilitating Stau1-mediated mRNA decay comprising
30 contacting with a system, wherein the system comprises the components for SMD such as, for example, an ALU element containing lncRNA, Upf1, Upf2, or Upf3. Also disclosed are methods of facilitating Stau1-mediated mRNA decay comprising contacting the SMD target

with Stau1 and/ an ALU element containing lncRNA. The combination of Stau1 and lncRNA can be simultaneous or sequential.

76. As described above, the compositions can also be administered *in vivo* in a pharmaceutically acceptable carrier. By “pharmaceutically acceptable” is meant a material
5 that is not biologically or otherwise undesirable, i.e., the material may be administered to a subject, along with the nucleic acid or vector, without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. The carrier would naturally be selected to minimize any degradation of the active ingredient and to minimize any adverse
10 side effects in the subject, as would be well known to one of skill in the art.

77. The compositions may be administered orally, parenterally (e.g., intravenously), by intramuscular injection, by intraperitoneal injection, transdermally, extracorporeally, topically or the like, although topical intranasal administration or administration by inhalant is typically preferred. As used herein, “topical intranasal administration” means delivery of the
15 compositions into the nose and nasal passages through one or both of the nares and can comprise delivery by a spraying mechanism or droplet mechanism, or through aerosolization of the nucleic acid or vector. The latter may be effective when a large number of animals is to be treated simultaneously. Administration of the compositions by inhalant can be through the nose or mouth via delivery by a spraying or droplet mechanism. Delivery can also be directly
20 to any area of the respiratory system (e.g., lungs) via intubation. The exact amount of the compositions required will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the severity of the allergic disorder being treated, the particular nucleic acid or vector used, its mode of administration and the like. Thus, it is not possible to specify an exact amount for every composition. However, an appropriate amount
25 can be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein.

78. Parenteral administration of the composition, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as
30 emulsions. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system such that a constant dosage is maintained. See, e.g., U.S. Patent No. 3,610,795, which is incorporated by reference herein.

79. The materials may be in solution, suspension (for example, incorporated into microparticles, liposomes, or cells). These may be targeted to a particular cell type via antibodies, receptors, or receptor ligands. The following references are examples of the use of this technology to target specific proteins to tumor tissue. Vehicles such as “stealth” and other antibody conjugated liposomes (including lipid mediated drug targeting to colonic carcinoma), receptor mediated targeting of DNA through cell specific ligands, lymphocyte directed tumor targeting, and highly specific therapeutic retroviral targeting of murine glioma cells *in vivo*. The following references are examples of the use of this technology to target specific proteins to tumor tissue. In general, receptors are involved in pathways of endocytosis, either constitutive or ligand induced. These receptors cluster in clathrin-coated pits, enter the cell via clathrin-coated vesicles, pass through an acidified endosome in which the receptors are sorted, and then either recycle to the cell surface, become stored intracellularly, or are degraded in lysosomes. The internalization pathways serve a variety of functions, such as nutrient uptake, removal of activated proteins, clearance of macromolecules, opportunistic entry of viruses and toxins, dissociation and degradation of ligand, and receptor-level regulation. Many receptors follow more than one intracellular pathway, depending on the cell type, receptor concentration, type of ligand, ligand valency, and ligand concentration. Molecular and cellular mechanisms of receptor-mediated endocytosis has been reviewed (Brown and Greene, *DNA and Cell Biology* 10:6, 399-409 (1991)).

80. The compositions, including antibodies, can be used therapeutically in combination with a pharmaceutically acceptable carrier.

81. Pharmaceutical carriers are known to those skilled in the art. These most typically would be standard carriers for administration of drugs to humans, including solutions such as sterile water, saline, and buffered solutions at physiological pH. The compositions can be administered intramuscularly or subcutaneously. Other compounds will be administered according to standard procedures used by those skilled in the art.

82. Pharmaceutical compositions may include carriers, thickeners, diluents, buffers, preservatives, surface active agents and the like in addition to the molecule of choice. Pharmaceutical compositions may also include one or more active ingredients such as antimicrobial agents, antiinflammatory agents, anesthetics, and the like.

83. The pharmaceutical composition may be administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated.

Administration may be topically (including ophthalmically, vaginally, rectally, intranasally), orally, by inhalation, or parenterally, for example by intravenous drip, subcutaneous, intraperitoneal or intramuscular injection. The disclosed antibodies or other agents can be administered intravenously, intraperitoneally, intramuscularly, subcutaneously, or
5 transdermally.

84. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous
10 solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials,
15 anti-oxidants, chelating agents, and inert gases and the like.

85. Formulations for topical administration may include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable.

86. Compositions for oral administration include powders or granules, suspensions or
20 solutions in water or non-aqueous media, capsules, sachets, or tablets. Thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders may be desirable.

87. Some of the compositions may potentially be administered as a pharmaceutically acceptable acid- or base- addition salt, formed by reaction with inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric
25 acid, and phosphoric acid, and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, and fumaric acid, or by reaction with an inorganic base such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, and organic bases such as mono-, di-, trialkyl and aryl amines and substituted ethanolamines.

88. The dosage ranges for the administration of the compositions are those large
30 enough to produce the desired effect in which the symptoms disorder are effected. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age,

condition, sex and extent of the disease in the patient and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of any counterindications. Dosage can vary, and can be administered in one or more dose administrations daily, for one or several days.

5 89. The disclosed methods and compositions can also be used for example as tools to isolate and test new drug candidates for a variety of diseases. They can also be used for the continued isolation and study, for example, SMD. Their use as exogenous DNA delivery devices can be expanded for nearly any reason desired by those of skill in the art.

10 90. The disclosed compositions can be used as targets for any combinatorial technique to identify molecules or macromolecular molecules that interact with the disclosed compositions in a desired way. The nucleic acids, peptides, and related molecules disclosed herein can be used as targets for the combinatorial approaches. Also disclosed are the compositions that are identified through combinatorial techniques or screening techniques in which the compositions disclosed in SEQ ID NOS:1, 2, or portions thereof,
15 are used as the target in a combinatorial or screening protocol.

 91. It is understood that when using the disclosed compositions in combinatorial techniques or screening methods, molecules, such as macromolecular molecules, will be identified that have particular desired properties such as inhibition or stimulation or the target molecule's function. The molecules identified and isolated when using the disclosed
20 compositions, such as, Stau1, are also disclosed. Thus, the products produced using the combinatorial or screening approaches that involve the disclosed compositions, such as, Stau1, are also considered herein disclosed.

 92. Combinatorial chemistry includes but is not limited to all methods for isolating small molecules or macromolecules that are capable of binding either a small molecule or
25 another macromolecule, typically in an iterative process. Proteins, oligonucleotides, and sugars are examples of macromolecules. For example, oligonucleotide molecules with a given function, catalytic or ligand-binding, can be isolated from a complex mixture of random oligonucleotides in what has been referred to as "in vitro genetics" (Szostak, *TIBS* 19:89, 1992). One synthesizes a large pool of molecules bearing random and defined
30 sequences and subjects that complex mixture, for example, approximately 10^{15} individual sequences in 100 μ g of a 100 nucleotide RNA, to some selection and enrichment process. Through repeated cycles of affinity chromatography and PCR amplification of the molecules bound to the ligand on the column, Ellington and Szostak (1990) estimated that 1

in 10^{10} RNA molecules folded in such a way as to bind a small molecule dyes. DNA molecules with such ligand-binding behavior have been isolated as well (Ellington and Szostak, 1992; Bock et al, 1992). Techniques aimed at similar goals exist for small organic molecules, proteins, antibodies and other macromolecules known to those of skill in the art.

5 Screening sets of molecules for a desired activity whether based on small organic libraries, oligonucleotides, or antibodies is broadly referred to as combinatorial chemistry.

Combinatorial techniques are particularly suited for defining binding interactions between molecules and for isolating molecules that have a specific binding activity, often called aptamers when the macromolecules are nucleic acids.

10 93. There are a number of methods for isolating proteins which either have de novo SMD activity or a modified activity. For example, phage display libraries have been used to isolate numerous peptides that interact with a specific target.

94. A preferred method for isolating proteins that have a given function is described by Roberts and Szostak (Roberts R.W. and Szostak J.W. Proc. Natl. Acad. Sci. USA, 15 94(23)12997-302 (1997). This combinatorial chemistry method couples the functional power of proteins and the genetic power of nucleic acids. An RNA molecule is generated in which a puromycin molecule is covalently attached to the 3'-end of the RNA molecule. An *in vitro* translation of this modified RNA molecule causes the correct protein, encoded by the RNA to be translated. In addition, because of the attachment of the puromycin, a 20 peptidyl acceptor which cannot be extended, the growing peptide chain is attached to the puromycin which is attached to the RNA. Thus, the protein molecule is attached to the genetic material that encodes it. Normal *in vitro* selection procedures can now be done to isolate functional peptides. Once the selection procedure for peptide function is complete traditional nucleic acid manipulation procedures are performed to amplify the nucleic acid 25 that codes for the selected functional peptides. After amplification of the genetic material, new RNA is transcribed with puromycin at the 3'-end, new peptide is translated and another functional round of selection is performed. Thus, protein selection can be performed in an iterative manner just like nucleic acid selection techniques. The peptide which is translated is controlled by the sequence of the RNA attached to the puromycin. This sequence can be 30 anything from a random sequence engineered for optimum translation (i.e. no stop codons etc.) or it can be a degenerate sequence of a known RNA molecule to look for improved or altered function of a known peptide. The conditions for nucleic acid amplification and *in vitro* translation are well known to those of ordinary skill in the art.

95. Another preferred method for combinatorial methods designed to isolate peptides is described in Cohen et al. (Cohen B.A., et al., Proc. Natl. Acad. Sci. USA 95(24):14272-7 (1998)). This method utilizes and modifies two-hybrid technology. Yeast two-hybrid systems are useful for the detection and analysis of protein:protein interactions.

5 The two-hybrid system, initially described in the yeast *Saccharomyces cerevisiae*, is a powerful molecular genetic technique for identifying new regulatory molecules, specific to the protein of interest. Cohen et al. modified this technology so that novel interactions between synthetic or engineered peptide sequences could be identified which bind a molecule of choice. The benefit of this type of technology is that the selection is done in an

10 intracellular environment. The method utilizes a library of peptide molecules that attached to an acidic activation domain.

96. Using methodology well known to those of skill in the art, in combination with various combinatorial libraries, one can isolate and characterize those small molecules or macromolecules, which bind to or interact with the desired target. The relative binding

15 affinity of these compounds can be compared and optimum compounds identified using competitive binding studies, which are well known to those of skill in the art.

97. As used herein combinatorial methods and libraries included traditional screening methods and libraries as well as methods and libraries used in interactive processes.

20 98. The disclosed compositions can be used as targets for any molecular modeling technique to identify either the structure of the disclosed compositions or to identify potential or actual molecules, such as small molecules, which interact in a desired way with the disclosed compositions. The nucleic acids, peptides, and related molecules disclosed herein can be used as targets in any molecular modeling program or approach.

25 99. It is understood that when using the disclosed compositions in modeling techniques, molecules, such as macromolecular molecules, will be identified that have particular desired properties such as inhibition or stimulation or the target molecule's function. The molecules identified and isolated when using the disclosed compositions, such as, Stau1 and Upf1, are also disclosed. Thus, the products produced using the

30 molecular modeling approaches that involve the disclosed compositions, such as, Stau1 and Upf1, are also considered herein disclosed.

100. Thus, one way to isolate molecules that bind a molecule of choice is through rational design. This is achieved through structural information and computer modeling.

Computer modeling technology allows visualization of the three-dimensional atomic structure of a selected molecule and the rational design of new compounds that will interact with the molecule. The three-dimensional construct typically depends on data from x-ray crystallographic analyses or NMR imaging of the selected molecule. The molecular dynamics require force field data. The computer graphics systems enable prediction of how a new compound will link to the target molecule and allow experimental manipulation of the structures of the compound and target molecule to perfect binding specificity.

Prediction of what the molecule-compound interaction will be when small changes are made in one or both requires molecular mechanics software and computationally intensive computers, usually coupled with user-friendly, menu-driven interfaces between the molecular design program and the user.

101. Examples of molecular modeling systems are the CHARMM and QUANTA programs, Polygen Corporation, Waltham, MA. CHARMM performs the energy minimization and molecular dynamics functions. QUANTA performs the construction, graphic modeling and analysis of molecular structure. QUANTA allows interactive construction, modification, visualization, and analysis of the behavior of molecules with each other.

102. Although described above with reference to design and generation of compounds which could alter binding, one could also screen libraries of known compounds, including natural products or synthetic chemicals, and biologically active materials, including proteins, for compounds which alter substrate binding or enzymatic activity.

103. The term “antibodies” is used herein in a broad sense and includes both polyclonal and monoclonal antibodies. In addition to intact immunoglobulin molecules, also included in the term “antibodies” are fragments or polymers of those immunoglobulin molecules, and human or humanized versions of immunoglobulin molecules or fragments thereof, as described herein. The antibodies are tested for their desired activity using the *in vitro* assays described herein, or by analogous methods, after which their *in vivo* therapeutic and/or prophylactic activities are tested according to known clinical testing methods.

104. The term “monoclonal antibody” as used herein refers to an antibody obtained from a substantially homogeneous population of antibodies, i.e., the individual antibodies within the population are identical except for possible naturally occurring mutations that may be present in a small subset of the antibody molecules. The monoclonal antibodies herein specifically include “chimeric” antibodies in which a portion of the heavy

and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, as long as they exhibit the desired antagonistic activity.

105. Monoclonal antibodies of the invention can be prepared using hybridoma methods, such as those described by Kohler and Milstein, *Nature*, 256:495 (1975). In a hybridoma method, a mouse or other appropriate host animal is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized *in vitro*, e.g., using the Stau1 or Upf1 described herein.

106. The monoclonal antibodies may also be made by recombinant DNA methods. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). Libraries of antibodies or active antibody fragments can also be generated and screened using phage display techniques.

107. *In vitro* methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly, Fab fragments, can be accomplished using routine techniques known in the art. For instance, digestion can be performed using papain. Papain digestion of antibodies typically produces two identical antigen binding fragments, called Fab fragments, each with a single antigen binding site, and a residual Fc fragment. Pepsin treatment yields a fragment that has two antigen combining sites and is still capable of cross-linking antigen.

108. The fragments, whether attached to other sequences or not, can also include insertions, deletions, substitutions, or other selected modifications of particular regions or specific amino acids residues, provided the activity of the antibody or antibody fragment is not significantly altered or impaired compared to the non-modified antibody or antibody fragment. These modifications can provide for some additional property, such as to remove/add amino acids capable of disulfide bonding, to increase its bio-longevity, to alter its secretory characteristics, etc. In any case, the antibody or antibody fragment must possess a bioactive property, such as specific binding to its cognate antigen. Functional or

active regions of the antibody or antibody fragment may be identified by mutagenesis of a specific region of the protein, followed by expression and testing of the expressed polypeptide. Such methods are readily apparent to a skilled practitioner in the art and can include site-specific mutagenesis of the nucleic acid encoding the antibody or antibody
5 fragment. (Zoller, M.J. *Curr. Opin. Biotechnol.* 3:348-354, 1992).

109. As used herein, the term “antibody” or “antibodies” can also refer to a human antibody and/or a humanized antibody. Many non-human antibodies (e.g., those derived from mice, rats, or rabbits) are naturally antigenic in humans, and thus can give rise to undesirable immune responses when administered to humans. Therefore, the use of
10 human or humanized antibodies in the methods of the invention serves to lessen the chance that an antibody administered to a human will evoke an undesirable immune response.

110. The human antibodies of the invention can be prepared using any technique. Human antibodies of the invention (and fragments thereof) can also be produced using phage display libraries.

15 111. The human antibodies of the invention can also be obtained from transgenic animals. For example, transgenic, mutant mice that are capable of producing a full repertoire of human antibodies, in response to immunization, have been described (see, e.g., Jakobovits et al., *Proc. Natl. Acad. Sci. USA*, 90:2551-255 (1993); Jakobovits et al., *Nature*, 362:255-258 (1993); Bruggermann et al., *Year in Immunol.*, 7:33 (1993)). Specifically, the
20 homozygous deletion of the antibody heavy chain joining region (J(H)) gene in these chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production, and the successful transfer of the human germ-line antibody gene array into such germ-line mutant mice results in the production of human antibodies upon antigen challenge.

25 112. Antibody humanization techniques generally involve the use of recombinant DNA technology to manipulate the DNA sequence encoding one or more polypeptide chains of an antibody molecule. Accordingly, a humanized form of a non-human antibody (or a fragment thereof) is a chimeric antibody or antibody chain (or a fragment thereof, such as an Fv, Fab, Fab', or other antigen-binding portion of an antibody) which contains a
30 portion of an antigen binding site from a non-human (donor) antibody integrated into the framework of a human (recipient) antibody.

113. To generate a humanized antibody, residues from one or more complementarity determining regions (CDRs) of a recipient (human) antibody molecule are

replaced by residues from one or more CDRs of a donor (non-human) antibody molecule that is known to have desired antigen binding characteristics (e.g., a certain level of specificity and affinity for the target antigen). In some instances, Fv framework (FR) residues of the human antibody are replaced by corresponding non-human residues.

5 Humanized antibodies may also contain residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from
10 analogous sites in rodent antibodies. Humanized antibodies generally contain at least a portion of an antibody constant region (Fc), typically that of a human antibody.

114. Methods for humanizing non-human antibodies are well known in the art. For example, humanized antibodies can be generated according to the methods of Winter and co-workers, by substituting rodent CDRs or CDR sequences for the corresponding
15 sequences of a human antibody.

115. Antibodies of the invention are preferably administered to a subject in a pharmaceutically acceptable carrier as described above. Effective dosages and schedules for administering the antibodies may be determined empirically, and making such determinations is within the skill in the art. Those skilled in the art will understand that the
20 dosage of antibodies that must be administered will vary depending on, for example, the subject that will receive the antibody, the route of administration, the particular type of antibody used and other drugs being administered. A typical daily dosage of the antibody used alone might range from about 1 µg/kg to up to 100 mg/kg of body weight or more per day, depending on the factors mentioned above.

25 116. Following administration of an antibody for treating, inhibiting, or preventing a condition, the efficacy of the therapeutic antibody can be assessed in various ways well known to the skilled practitioner. Specifically, SMD can be assessed directly or indirectly as taught herein.

117. In the methods described above which include the administration and uptake
30 of exogenous DNA into the cells of a subject (i.e., gene transduction or transfection), the nucleic acids of the present invention can be in the form of naked DNA or RNA, or the nucleic acids can be in a vector for delivering the nucleic acids to the cells, whereby the antibody-encoding DNA fragment is under the transcriptional regulation of a promoter, as

would be well understood by one of ordinary skill in the art. The vector can be a commercially available preparation, such as an adenovirus vector (Quantum Biotechnologies, Inc. (Laval, Quebec, Canada). Delivery of the nucleic acid or vector to cells can be via a variety of mechanisms. As one example, delivery can be via a liposome, using commercially available liposome preparations such as LIPOFECTIN, LIPOFECTAMINE (GIBCO-BRL, Inc., Gaithersburg, MD), SUPERFECT (Qiagen, Inc. Hilden, Germany) and TRANSFECTAM (Promega Biotec, Inc., Madison, WI), as well as other liposomes developed according to procedures standard in the art. In addition, the nucleic acid or vector of this invention can be delivered *in vivo* by electroporation, the technology for which is available from Genetronics, Inc. (San Diego, CA) as well as by means of a SONOPORATION machine (ImaRx Pharmaceutical Corp., Tucson, AZ).

118. As one example, vector delivery can be via a viral system, such as a retroviral vector system which can package a recombinant retroviral genome. The recombinant retrovirus can then be used to infect and thereby deliver to the infected cells nucleic acid encoding a broadly neutralizing antibody (or active fragment thereof) of the invention. The exact method of introducing the altered nucleic acid into mammalian cells is, of course, not limited to the use of retroviral vectors. Other techniques are widely available for this procedure including the use of adenoviral vectors, adeno-associated viral (AAV) vectors, lentiviral vectors, pseudotyped retroviral vectors. Physical transduction techniques can also be used, such as liposome delivery and receptor-mediated and other endocytosis mechanisms. This invention can be used in conjunction with any of these or other commonly used gene transfer methods.

119. As one example, if the antibody-encoding nucleic acid of the invention is delivered to the cells of a subject in an adenovirus vector, the dosage for administration of adenovirus to humans can range from about 10^7 to 10^9 plaque forming units (pfu) per injection but can be as high as 10^{12} pfu per injection. A subject can receive a single injection, or, if additional injections are necessary, they can be repeated at six month intervals (or other appropriate time intervals, as determined by the skilled practitioner) for an indefinite period and/or until the efficacy of the treatment has been established.

120. Parenteral administration of the nucleic acid or vector of the present invention, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution of suspension in liquid prior to injection, or as emulsions. A more recently revised

approach for parenteral administration involves use of a slow release or sustained release system such that a constant dosage is maintained. See, e.g., U.S. Patent No. 3,610,795, which is incorporated by reference herein. For additional discussion of suitable formulations and various routes of administration of therapeutic compounds.

5 **Compositions**

121. The disclosed screening and treatment methods make use of agents administered to a subject to achieve a desired effect. It is understood and herein contemplated that “agent” can refer to any compound, functional nucleic acid, siRNA, peptide, protein, antibody, or small molecule. Thus, for example, one embodiment of the
10 disclosed methods is a method of treating a subject with an agent wherein the substance is Stau1, an ALU element-containing lncRNA that base pairs with the ALU element in the mRNA of the 3' UTR of the truncated or erroneous protein, or a complex comprising one or more of an ALU element-containing lncRNA, Stau1 Upf1, Upf2, and Upf3. Thus, for example, disclosed It is understood that by “SMD complex” is any combination of one or
15 more of the essential components of SMD. Such administration can be direct or indirect. For example, the Stau1 can be administered directly or by transferr with a subject with a Stau1 encoding nucleic acid or by administering to the subject a compound that modulates SMD.

122. An agent that modulates SMD can be a nucleic acid. Therefore specifically
20 disclosed and herein contemplated is an agent, wherein the agent is a vector comprising a nucleic acid that encodes an SMD modulator. Also disclosed is a vector comprising a nucleic acid that encodes an SMD modulator. Also disclosed is a cell comprising the disclosed vectors.

123. Thus specifically contemplated and disclosed herein is an agent that
25 modulates SMD, wherein the substance is an siRNA that modulates SMD. It is understood that the siRNA can bind any factor that modulates SMD. For example, specifically disclosed is an siRNA, wherein the siRNA binds Upf1. Also disclosed is a substance comprising siRNA, wherein the siRNA binds Stau1. It is further understood that providing additional components of SMD can facilitate increased SMD activity. For example,
30 wherein the agent is Stau1, additional Stau1 will be available to bind to the SBS. Additionally, wherein the agent is an ALU element-containing lncRNA, an additional component of the SBS is provided to bind with the ALU element in the 3' UTR of the SMD

target. Thus disclosed herein are agents, wherein the agent is Stau1, an lncRNA, or an siRNA that binds to an ALU element.

124. Disclosed are the components to be used to prepare the disclosed compositions as well as the compositions themselves to be used within the methods disclosed herein. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutation of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a particular Stau1 or ALU element is disclosed and discussed and a number of modifications that can be made to a number of molecules including the Stau1 or the ALU element are discussed, specifically contemplated is each and every combination and permutation of Stau1 and the ALU element and the modifications that are possible unless specifically indicated to the contrary. Thus, if a class of molecules A, B, and C are disclosed as well as a class of molecules D, E, and F and an example of a combination molecule, A-D is disclosed, then even if each is not individually recited each is individually and collectively contemplated meaning combinations, A-E, A-F, B-D, B-E, B-F, C-D, C-E, and C-F are considered disclosed. Likewise, any subset or combination of these is also disclosed. Thus, for example, the sub-group of A-E, B-F, and C-E would be considered disclosed. This concept applies to all aspects of this application including, but not limited to, steps in methods of making and using the disclosed compositions. Thus, if there are a variety of additional steps that can be performed it is understood that each of these additional steps can be performed with any specific embodiment or combination of embodiments of the disclosed methods.

125. As used throughout, when reference is made to a particular protein or nucleic acid, some variation in amino acid or nucleotide sequence is expected without a substantial decline in function. Thus, Stau1 can include proteins or nucleic acid sequences having at least 80%, 85%, 90%, or 95% identity to the sequence set forth in SEQ ID NO: 1 or 2, or fragment thereof. Also disclosed are methods of the invention, wherein the ALU element of the lncRNA has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% identity to the sequence set forth in SEQ ID NO: 3, or fragment thereof.

126. It is understood that one way to define any known variants and derivatives or those that might arise, of the disclosed genes and proteins herein is through defining the variants and derivatives in terms of homology to specific known sequences. For example

SEQ ID NO: 1 sets forth a particular sequence of a Stau1 encoding nucleic acid, and SEQ ID NO: 2 sets forth a particular sequence of the protein encoded by SEQ ID NO: 1, an Stau1 protein. Specifically disclosed are variants of these and other genes and proteins herein disclosed which have at least, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 percent homology to the stated sequence. Those of skill in the art readily understand how to determine the homology of two proteins or nucleic acids, such as genes. For example, the homology can be calculated after aligning the two sequences so that the homology is at its highest level.

127. Another way of calculating homology can be performed by published algorithms. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman *Adv. Appl. Math.* 2: 482 (1981), by the homology alignment algorithm of Needleman and Wunsch, *J. Mol. Biol.* 48: 443 (1970), by the search for similarity method of Pearson and Lipman, *Proc. Natl. Acad. Sci. U.S.A.* 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection.

128. The same types of homology can be obtained for nucleic acids by for example the algorithms disclosed in Zuker, M. *Science* 244:48-52, 1989, Jaeger et al. *Proc. Natl. Acad. Sci. USA* 86:7706-7710, 1989, Jaeger et al. *Methods Enzymol.* 183:281-306, 1989 which are herein incorporated by reference for at least material related to nucleic acid alignment.

129. There are a variety of molecules disclosed herein that are nucleic acid based, including for example the nucleic acids that encode, for example Stau1 and Upf1, as well as various functional nucleic acids. The disclosed nucleic acids are made up of, for example, nucleotides, nucleotide analogs, or nucleotide substitutes. Non-limiting examples of these and other molecules are discussed herein. It is understood that for example, when a vector is expressed in a cell, that the expressed mRNA will typically be made up of A, C, G, and U. Likewise, it is understood that if, for example, an antisense molecule is introduced into a cell or cell environment through for example exogenous delivery, it is advantageous that the antisense molecule be made up of nucleotide analogs that reduce the degradation of the antisense molecule in the cellular environment.

130. A nucleotide is a molecule that contains a base moiety, a sugar moiety and a phosphate moiety. Nucleotides can be linked together through their phosphate moieties and

sugar moieties creating an internucleoside linkage. The base moiety of a nucleotide can be adenin-9-yl (A), cytosin-1-yl (C), guanin-9-yl (G), uracil-1-yl (U), and thymine-1-yl (T).

The sugar moiety of a nucleotide is a ribose or a deoxyribose. The phosphate moiety of a nucleotide is pentavalent phosphate. An non-limiting example of a nucleotide would be 3'-

5 AMP (3'-adenosine monophosphate) or 5'-GMP (5'-guanosine monophosphate).

131. A nucleotide analog is a nucleotide which contains some type of modification to either the base, sugar, or phosphate moieties. Modifications to the base moiety would include natural and synthetic modifications of A, C, G, and T/U as well as different purine or pyrimidine bases, such as uracil-5-yl (.psi.), hypoxanthin-9-yl (I), and 10 2-aminoadenin-9-yl. A modified base includes but is not limited to 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil 15 (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Additional base modifications can be found for example in U.S. Pat. 20 No. 3,687,808, Englisch et al., *Angewandte Chemie, International Edition*, 1991, 30, 613, and Sanghvi, Y. S., Chapter 15, *Antisense Research and Applications*, pages 289-302, Croke, S. T. and Lebleu, B. ed., CRC Press, 1993. Certain nucleotide analogs, such as 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 25 5-methylcytosine can increase the stability of duplex formation. Often time base modifications can be combined with for example a sugar modification, such as 2'-O-methoxyethyl, to achieve unique properties such as increased duplex stability.

132. Nucleotide analogs can also include modifications of the sugar moiety. Modifications to the sugar moiety would include natural modifications of the ribose and 30 deoxy ribose as well as synthetic modifications. Sugar modifications include but are not limited to the following modifications at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S-or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C₁ to C₁₀, alkyl or C₂ to C₁₀ alkenyl and

alkynyl. 2' sugar modifications also include but are not limited to $-O[(CH_2)_n O]_m CH_3$, $-O(CH_2)_n OCH_3$, $-O(CH_2)_n NH_2$, $-O(CH_2)_n CH_3$, $-O(CH_2)_n -ONH_2$, and $-O(CH_2)_n ON[(CH_2)_n CH_3]_2$, where n and m are from 1 to about 10.

133. Other modifications at the 2' position include but are not limited to: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3 , OCN, Cl, Br, CN, CF_3 , OCF_3 , $SOCH_3$, $SO_2 CH_3$, ONO_2 , NO_2 , N_3 , NH_2 , heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. Similar modifications may also be made at other positions on the sugar, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Modified sugars would also include those that contain modifications at the bridging ring oxygen, such as CH_2 and S. Nucleotide sugar analogs may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar.

134. Nucleotide analogs can also be modified at the phosphate moiety. Modified phosphate moieties include but are not limited to those that can be modified so that the linkage between two nucleotides contains a phosphorothioate, chiral phosphorothioate, phosphorodithioate, phosphotriester, aminoalkylphosphotriester, methyl and other alkyl phosphonates including 3'-alkylene phosphonate and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates. It is understood that these phosphate or modified phosphate linkage between two nucleotides can be through a 3'-5' linkage or a 2'-5' linkage, and the linkage can contain inverted polarity such as 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

135. It is understood that nucleotide analogs need only contain a single modification, but may also contain multiple modifications within one of the moieties or between different moieties.

136. Nucleotide substitutes are molecules having similar functional properties to nucleotides, but which do not contain a phosphate moiety, such as peptide nucleic acid (PNA). Nucleotide substitutes are molecules that will recognize nucleic acids in a Watson-

Crick or Hoogsteen manner, but which are linked together through a moiety other than a phosphate moiety. Nucleotide substitutes are able to conform to a double helix type structure when interacting with the appropriate target nucleic acid.

137. Nucleotide substitutes are nucleotides or nucleotide analogs that have had the phosphate moiety and/or sugar moieties replaced. Nucleotide substitutes do not contain a standard phosphorus atom. Substitutes for the phosphate can be for example, short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH₂ component parts.

138. It is also possible to link other types of molecules (conjugates) to nucleotides or nucleotide analogs to enhance for example, cellular uptake. Conjugates can be chemically linked to the nucleotide or nucleotide analogs. Such conjugates include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989, 86, 6553-6556), cholic acid (Manoharan et al., Bioorg. Med. Chem. Lett., 1994, 4, 1053-1060), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., Ann. N.Y. Acad. Sci., 1992, 660, 306-309; Manoharan et al., Bioorg. Med. Chem. Lett., 1993, 3, 2765-2770), a thiocholesterol (Oberhauser et al., Nucl. Acids Res., 1992, 20, 533-538), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., EMBO J., 1991, 10, 1111-1118; Kabanov et al., FEBS Lett., 1990, 259, 327-330; Svinarchuk et al., Biochimie, 1993, 75, 49-54), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654; Shea et al., Nucl. Acids Res., 1990, 18, 3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., Nucleosides & Nucleotides, 1995, 14, 969-973), or adamantane acetic acid (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654), a palmityl moiety (Mishra et al., Biochim. Biophys. Acta, 1995, 1264, 229-237), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., J. Pharmacol. Exp. Ther., 1996, 277, 923-937).

139. There are a variety of sequences related to the Stau1 and Upf1 gene having the following Genbank Accession Numbers: BC050432 and NM_002911, respectively. These sequences and others are herein incorporated by reference in their entireties as well as for individual subsequences contained therein.

5 140. One particular sequence set forth in SEQ ID NO: 1 and having Genbank accession number BC050432 is used herein, as an example, to exemplify the disclosed compositions and methods. It is understood that the description related to this sequence is applicable to any sequence related to Stau1 unless specifically indicated otherwise. Those of skill in the art understand how to resolve sequence discrepancies and differences and to
10 adjust the compositions and methods relating to a particular sequence to other related sequences (i.e. sequences of Upf1, SMD targets, ALU elements, ALU element containing lncRNA). Primers and/or probes can be designed for any Stau1 or Upf1 sequence given the information disclosed herein and known in the art.

 141. Functional nucleic acids are nucleic acid molecules that have a specific
15 function, such as binding a target molecule or catalyzing a specific reaction. Functional nucleic acid molecules can be divided into the following categories, which are not meant to be limiting. For example, functional nucleic acids include antisense molecules, aptamers, ribozymes, triplex forming molecules, and external guide sequences. The functional nucleic acid molecules can act as effectors, inhibitors, modulators, and stimulators of a
20 specific activity possessed by a target molecule, or the functional nucleic acid molecules can possess a de novo activity independent of any other molecules.

 142. Antisense molecules are designed to interact with a target nucleic acid molecule through either canonical or non-canonical base pairing. The interaction of the antisense molecule and the target molecule is designed to promote the destruction of the
25 target molecule through, for example, RNaseH mediated RNA-DNA hybrid degradation. Alternatively the antisense molecule is designed to interrupt a processing function that normally would take place on the target molecule, such as transcription or replication. Antisense molecules can be designed based on the sequence of the target molecule. Numerous methods for optimization of antisense efficiency by finding the most accessible
30 regions of the target molecule exist. Exemplary methods would be in vitro selection experiments and DNA modification studies using DMS and DEPC. It is preferred that antisense molecules bind the target molecule with a dissociation constant (k_d) less than 10^{-6} . It is more preferred that antisense molecules bind with a k_d less than 10^{-8} . It is also more

preferred that the antisense molecules bind the target molecule with a k_d less than 10^{-10} . It is also preferred that the antisense molecules bind the target molecule with a k_d less than 10^{-12} .

143. Aptamers are molecules that interact with a target molecule, preferably in a specific way. Typically aptamers are small nucleic acids ranging from 15-50 bases in length that fold into defined secondary and tertiary structures, such as stem-loops or G-quartets. Aptamers can bind small molecules, such as ATP and theophylline, as well as large molecules, such as reverse transcriptase and thrombin. Aptamers can bind very tightly with k_d s from the target molecule of less than 10^{-12} M. It is preferred that the aptamers bind the target molecule with a k_d less than 10^{-6} . It is more preferred that the aptamers bind the target molecule with a k_d less than 10^{-8} . It is also more preferred that the aptamers bind the target molecule with a k_d less than 10^{-10} . It is also preferred that the aptamers bind the target molecule with a k_d less than 10^{-12} . Aptamers can bind the target molecule with a very high degree of specificity. For example, aptamers have been isolated that have greater than a 10000 fold difference in binding affinities between the target molecule and another molecule that differ at only a single position on the molecule (United States patent 5,543,293). It is preferred that the aptamer have a k_d with the target molecule at least 10 fold lower than the k_d with a background binding molecule. It is more preferred that the aptamer have a k_d with the target molecule at least 100 fold lower than the k_d with a background binding molecule. It is more preferred that the aptamer have a k_d with the target molecule at least 1000 fold lower than the k_d with a background binding molecule. It is preferred that the aptamer have a k_d with the target molecule at least 10000 fold lower than the k_d with a background binding molecule. It is preferred when doing the comparison for a polypeptide for example, that the background molecule be a different polypeptide.

144. It is understood herein that "siRNA" refers to double-stranded RNAs that can induce sequence-specific post-transcriptional gene silencing, thereby decreasing or even inhibiting gene expression. In one example, an siRNA triggers the specific degradation of homologous RNA molecules, such as mRNAs, within the region of sequence identity between both the siRNA and the target RNA. For example, WO 02/44321 discloses siRNAs capable of sequence-specific degradation of target mRNAs when base-paired with 3' overhanging ends. The direction of dsRNA processing determines whether a sense or an antisense target RNA can be cleaved by the produced siRNA endonuclease complex. Thus, siRNAs can be used to modulate transcription, for example, by silencing genes such as

Stau1 and Upf1. The effects of siRNAs have been demonstrated in cells from a variety of organisms, including *Drosophila*, *C. elegans*, insects, frogs, plants, fungi, mice and humans.

In certain examples, siRNAs are directed against certain target genes to down regulate gene expression. For example, Stau1, ALU element containing lncRNA, SMD targets, or Upf1
5 expression can be down regulated by specifically targeting the siRNA to Stau1 or Upf1.

145. As discussed herein there are numerous variants of the Stau1 protein and Upf1 protein that are known and herein contemplated. In addition, to the known functional Stau1 and Upf1 strain variants, there are derivatives of the Stau1 and Upf1 proteins which also function in the disclosed methods and compositions. Protein variants and derivatives

10 are well understood to those of skill in the art and in can involve amino acid sequence modifications. For example, amino acid sequence modifications typically fall into one or more of three classes: substitutional, insertional or deletional variants. Insertions include amino and/or carboxyl terminal fusions as well as intrasequence insertions of single or multiple amino acid residues. Insertions ordinarily will be smaller insertions than those of
15 amino or carboxyl terminal fusions, for example, on the order of one to four residues.

Immunogenic fusion protein derivatives, such as those described in the examples, are made by fusing a polypeptide sufficiently large to confer immunogenicity to the target sequence by cross-linking in vitro or by recombinant cell culture transformed with DNA encoding the fusion. Deletions are characterized by the removal of one or more amino acid residues from
20 the protein sequence. Typically, no more than about from 2 to 6 residues are deleted at any one site within the protein molecule. These variants ordinarily are prepared by site specific mutagenesis of nucleotides in the DNA encoding the protein, thereby producing DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture.

Techniques for making substitution mutations at predetermined sites in DNA having a

25 known sequence are well known, for example M13 primer mutagenesis and PCR mutagenesis. Amino acid substitutions are typically of single residues, but can occur at a number of different locations at once; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. Deletions or insertions preferably are made in adjacent pairs, i.e. a deletion of 2 residues or insertion
30 of 2 residues. Substitutions, deletions, insertions or any combination thereof may be combined to arrive at a final construct. The mutations must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. Substitutional variants are those in which at least one residue

has been removed and a different residue inserted in its place. Such substitutions generally are made in accordance with the following Tables 1 and 2 and are referred to as conservative substitutions.

146. TABLE 1:Amino Acid Abbreviations

Amino Acid	Abbreviations
alanine	Ala; A
arginine	Arg; R
asparagine	Asn; N
aspartic acid	Asp; D
cysteine	Cys; C
glutamic acid	Glu; E
glutamine	Gln; Q
glycine	Gly; G
histidine	His; H
isoleucine	Ile; I
leucine	Leu; L
lysine	Lys; K
methionine	Met; M
phenylalanine	Phe; F
proline	Pro; P
serine	Ser; S
threonine	Thr; T
tyrosine	Tyr; Y
tryptophan	Trp; W
valine	Val; V

5

TABLE 2:Amino Acid Substitutions
Original Residue Exemplary Conservative Substitutions, others are known in the art.
Ala; Ser
Arg; Lys, Gln
Asn; Gln; His
Asp; Glu
Cys; Ser
Gln; Asn; Lys
Glu; Asp
Gly; Pro
His; Asn; Gln
Ile; Leu; Val
Leu; Ile; Val
Lys; Arg; Gln;
Met; Leu; Ile
Phe; Met; Leu; Tyr
Ser; Thr
Thr; Ser

Trp; Tyr
Tyr; Trp; Phe
Val; Ile; Leu

147. Substantial changes in function or immunological identity are made by selecting substitutions that are less conservative than those in Table 2, i.e., selecting residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site or (c) the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in the protein properties will be those in which (a) a hydrophilic residue, e.g. seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g. leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine, in this case, (e) by increasing the number of sites for sulfation and/or glycosylation.

148. For example, the replacement of one amino acid residue with another that is biologically and/or chemically similar is known to those skilled in the art as a conservative substitution. For example, a conservative substitution would be replacing one hydrophobic residue for another, or one polar residue for another. The substitutions include combinations such as, for example, Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr. Such conservatively substituted variations of each explicitly disclosed sequence are included within the mosaic polypeptides provided herein.

149. Substitutional or deletional mutagenesis can be employed to insert sites for N-glycosylation (Asn-X-Thr/Ser) or O-glycosylation (Ser or Thr). Deletions of cysteine or other labile residues also may be desirable. Deletions or substitutions of potential proteolysis sites, e.g. Arg, is accomplished for example by deleting one of the basic residues or substituting one by glutaminyl or histidyl residues.

150. Certain post-translational derivatizations are the result of the action of recombinant host cells on the expressed polypeptide. Glutaminyl and asparaginyl residues are frequently post-translationally deamidated to the corresponding glutamyl and aspartyl residues. Alternatively, these residues are deamidated under mildly acidic conditions.

Other post-translational modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the o-amino groups of lysine, arginine, and histidine side chains (T.E. Creighton, *Proteins: Structure and Molecular Properties*, W. H. Freeman & Co., San Francisco pp 79-86 [1983]),
5 acetylation of the N-terminal amine and, in some instances, amidation of the C-terminal carboxyl.

151. It is understood that one way to define the variants and derivatives of the disclosed proteins herein is through defining the variants and derivatives in terms of homology/identity to specific known sequences. For example, SEQ ID NO: 2 sets forth a
10 particular sequence of Stau1. Specifically disclosed are variants of these and other proteins herein disclosed which have at least, 70% or 75% or 80% or 85% or 90% or 95% homology to the stated sequence. Those of skill in the art readily understand how to determine the homology of two proteins. For example, the homology can be calculated after aligning the two sequences so that the homology is at its highest level.

152. Another way of calculating homology can be performed by published
15 algorithms. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman *Adv. Appl. Math.* 2: 482 (1981), by the homology alignment algorithm of Needleman and Wunsch, *J. Mol. Biol.* 48: 443 (1970), by the search for similarity method of Pearson and Lipman, *Proc. Natl. Acad. Sci. U.S.A.* 85:
20 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection.

153. It is understood that the description of conservative mutations and homology can be combined together in any combination, such as embodiments that have at least 70%
25 homology to a particular sequence wherein the variants are conservative mutations.

154. As this specification discusses various proteins and protein sequences it is understood that the nucleic acids that can encode those protein sequences are also disclosed. This would include all degenerate sequences related to a specific protein sequence, i.e. all nucleic acids having a sequence that encodes one particular protein sequence as well as all
30 nucleic acids, including degenerate nucleic acids, encoding the disclosed variants and derivatives of the protein sequences. Thus, while each particular nucleic acid sequence may not be written out herein, it is understood that each and every sequence is in fact disclosed and described herein through the disclosed protein sequence. For example, one of the many

nucleic acid sequences that can encode the protein sequence set forth in SEQ ID NO:2 is set forth in SEQ ID NO:1. It is understood that all of the nucleic acid sequences that encode this particular derivative of the Stau1 are also disclosed including for example SEQ ID NO:1 which set forth two of the degenerate nucleic acid sequences that encode the particular polypeptide set forth in SEQ ID NO:2. It is also understood that while no amino acid sequence indicates what particular DNA sequence encodes that protein within an organism, where particular variants of a disclosed protein are disclosed herein, the known nucleic acid sequence that encodes that protein in the particular Stau1 from which that protein arises is also known and herein disclosed and described.

10 155. There are a number of compositions and methods which can be used to deliver nucleic acids to cells, either in vitro or in vivo. These methods and compositions can largely be broken down into two classes: viral based delivery systems and non-viral based delivery systems. For example, the nucleic acids can be delivered through a number of direct delivery systems such as, electroporation, lipofection, calcium phosphate precipitation, plasmids, viral vectors, viral nucleic acids, phage nucleic acids, phages, cosmids, or via transfer of genetic material in cells or carriers such as cationic liposomes. Appropriate means for transfection, including viral vectors, chemical transfectants, or physico-mechanical methods such as electroporation and direct diffusion of DNA, are described by, for example, Wolff, J. A., et al., *Science*, 247, 1465-1468, (1990); and Wolff, J. A. *Nature*, 352, 815-818, (1991) Such methods are well known in the art and readily adaptable for use with the compositions and methods described herein. In certain cases, the methods will be modified to specifically function with large DNA molecules. Further, these methods can be used to target certain diseases and cell populations by using the targeting characteristics of the carrier.

25 156. Transfer vectors can be any nucleotide construction used to deliver genes into cells (e.g., a plasmid), or as part of a general strategy to deliver genes, e.g., as part of recombinant retrovirus or adenovirus.

 157. As used herein, plasmid or viral vectors are agents that transport the disclosed nucleic acids, such as Stau1 and Upf1 into the cell without degradation and include a promoter yielding expression of the gene in the cells into which it is delivered. Viral vectors are, for example, Adenovirus, Adeno-associated virus, Herpes virus, Vaccinia virus, Polio virus, AIDS virus, neuronal trophic virus, Sindbis and other RNA viruses, including these viruses with the HIV backbone. Also preferred are any viral families which

share the properties of these viruses which make them suitable for use as vectors.

Retroviruses include Murine Maloney Leukemia virus, MMLV, and retroviruses that express the desirable properties of MMLV as a vector. Retroviral vectors are able to carry a larger genetic payload, i.e., a transgene or marker gene, than other viral vectors, and for this reason are a commonly used vector. However, they are not as useful in non-proliferating cells. Adenovirus vectors are relatively stable and easy to work with, have high titers, and can be delivered in aerosol formulation, and can transfect non-dividing cells. Pox viral vectors are large and have several sites for inserting genes, they are thermostable and can be stored at room temperature. A preferred embodiment is a viral vector which has been engineered so as to suppress the immune response of the host organism, elicited by the viral antigens. Preferred vectors of this type will carry coding regions for Interleukin 8 or 10.

158. Viral vectors can have higher transaction (ability to introduce genes) abilities than chemical or physical methods to introduce genes into cells. Typically, viral vectors contain, nonstructural early genes, structural late genes, an RNA polymerase III transcript, inverted terminal repeats necessary for replication and encapsidation, and promoters to control the transcription and replication of the viral genome. When engineered as vectors, viruses typically have one or more of the early genes removed and a gene or gene/promotor cassette is inserted into the viral genome in place of the removed viral DNA. Constructs of this type can carry up to about 8 kb of foreign genetic material. The necessary functions of the removed early genes are typically supplied by cell lines which have been engineered to express the gene products of the early genes in trans.

159. A retrovirus is an animal virus belonging to the virus family of Retroviridae, including any types, subfamilies, genus, or tropisms. A retrovirus is essentially a package which has packed into it nucleic acid cargo. The nucleic acid cargo carries with it a packaging signal, which ensures that the replicated daughter molecules will be efficiently packaged within the package coat. In addition to the package signal, there are a number of molecules which are needed in cis, for the replication, and packaging of the replicated virus. Typically a retroviral genome, contains the gag, pol, and env genes which are involved in the making of the protein coat. It is the gag, pol, and env genes which are typically replaced by the foreign DNA that it is to be transferred to the target cell. Retrovirus vectors typically contain a packaging signal for incorporation into the package coat, a sequence which signals the start of the gag transcription unit, elements necessary for reverse transcription, including a primer binding site to bind the tRNA primer of reverse

transcription, terminal repeat sequences that guide the switch of RNA strands during DNA synthesis, a purine rich sequence 5' to the 3' LTR that serve as the priming site for the synthesis of the second strand of DNA synthesis, and specific sequences near the ends of the LTRs that enable the insertion of the DNA state of the retrovirus to insert into the host genome. The removal of the gag, pol, and env genes allows for about 8 kb of foreign sequence to be inserted into the viral genome, become reverse transcribed, and upon replication be packaged into a new retroviral particle. This amount of nucleic acid is sufficient for the delivery of a one to many genes depending on the size of each transcript. It is preferable to include either positive or negative selectable markers along with other genes in the insert.

160. Since the replication machinery and packaging proteins in most retroviral vectors have been removed (gag, pol, and env), the vectors are typically generated by placing them into a packaging cell line. A packaging cell line is a cell line which has been transfected or transformed with a retrovirus that contains the replication and packaging machinery, but lacks any packaging signal. When the vector carrying the DNA of choice is transfected into these cell lines, the vector containing the gene of interest is replicated and packaged into new retroviral particles, by the machinery provided in cis by the helper cell. The genomes for the machinery are not packaged because they lack the necessary signals.

161. The benefit of the use of these viruses as vectors is that they are limited in the extent to which they can spread to other cell types, since they can replicate within an initial infected cell, but are unable to form new infectious viral particles. Recombinant adenoviruses have been shown to achieve high efficiency gene transfer after direct, in vivo delivery to airway epithelium, hepatocytes, vascular endothelium, CNS parenchyma and a number of other tissue sites. Recombinant adenoviruses achieve gene transduction by binding to specific cell surface receptors, after which the virus is internalized by receptor-mediated endocytosis, in the same manner as wild type or replication-defective adenovirus.

162. A viral vector can be one based on an adenovirus which has had the E1 gene removed and these virions are generated in a cell line such as the human 293 cell line. In another preferred embodiment both the E1 and E3 genes are removed from the adenovirus genome.

163. Another type of viral vector is based on an adeno-associated virus (AAV). This defective parvovirus is a preferred vector because it can infect many cell types and is

nonpathogenic to humans. AAV type vectors can transport about 4 to 5 kb and wild type AAV is known to stably insert into chromosome 19. Vectors which contain this site specific integration property are preferred. An especially preferred embodiment of this type of vector is the P4.1 C vector produced by Avigen, San Francisco, CA, which can contain
5 the herpes simplex virus thymidine kinase gene, HSV-tk, and/or a marker gene, such as the gene encoding the green fluorescent protein, GFP.

164. In another type of AAV virus, the AAV contains a pair of inverted terminal repeats (ITRs) which flank at least one cassette containing a promoter which directs cell-specific expression operably linked to a heterologous gene. Heterologous in this context
10 refers to any nucleotide sequence or gene which is not native to the AAV or B19 parvovirus.

165. Typically the AAV and B19 coding regions have been deleted, resulting in a safe, noncytotoxic vector. The AAV ITRs, or modifications thereof, confer infectivity and site-specific integration, but not cytotoxicity, and the promoter directs cell-specific
15 expression.

166. The vectors of the present invention thus provide DNA molecules which are capable of integration into a mammalian chromosome without substantial toxicity.

167. The inserted genes in viral and retroviral usually contain promoters, and/or enhancers to help control the expression of the desired gene product. A promoter is
20 generally a sequence or sequences of DNA that function when in a relatively fixed location in regard to the transcription start site. A promoter contains core elements required for basic interaction of RNA polymerase and transcription factors, and may contain upstream elements and response elements.

168. Molecular genetic experiments with large human herpesviruses have
25 provided a means whereby large heterologous DNA fragments can be cloned, propagated and established in cells permissive for infection with herpesviruses (Sun et al., Nature genetics 8: 33-41, 1994; Cotter and Robertson, Curr Opin Mol Ther 5: 633-644, 1999). These large DNA viruses (herpes simplex virus (HSV) and Epstein-Barr virus (EBV), have the potential to deliver fragments of human heterologous DNA > 150 kb to specific cells.
30 EBV recombinants can maintain large pieces of DNA in the infected B-cells as episomal DNA. Individual clones carried human genomic inserts up to 330 kb appeared genetically stable. The maintenance of these episomes requires a specific EBV nuclear protein, EBNA1, constitutively expressed during infection with EBV. Additionally, these vectors can be used

for transfection, where large amounts of protein can be generated transiently *in vitro*. Herpesvirus amplicon systems are also being used to package pieces of DNA > 220 kb and to infect cells that can stably maintain DNA as episomes.

169. Other useful systems include, for example, replicating and host-restricted
5 non-replicating vaccinia virus vectors.

170. The disclosed compositions can be delivered to the target cells in a variety of ways. For example, the compositions can be delivered through electroporation, or through lipofection, or through calcium phosphate precipitation. The delivery mechanism chosen will depend in part on the type of cell targeted and whether the delivery is occurring for
10 example *in vivo* or *in vitro*.

171. Thus, the compositions can comprise, lipids such as liposomes, such as cationic liposomes (e.g., DOTMA, DOPE, DC-cholesterol) or anionic liposomes. Liposomes can further comprise proteins to facilitate targeting a particular cell, if desired. Administration of a composition comprising a compound and a cationic liposome can be
15 administered to the blood afferent to a target organ or inhaled into the respiratory tract to target cells of the respiratory tract. Furthermore, the compound can be administered as a component of a microcapsule that can be targeted to specific cell types, such as macrophages, or where the diffusion of the compound or delivery of the compound from the microcapsule is designed for a specific rate or dosage.

20 172. In the methods described above which include the administration and uptake of exogenous DNA into the cells of a subject (i.e., gene transduction or transfection), delivery of the compositions to cells can be via a variety of mechanisms. As one example, delivery can be via a liposome, using commercially available liposome preparations such as LIPOFECTIN, LIPOFECTAMINE (GIBCO-BRL, Inc., Gaithersburg, MD), SUPERFECT
25 (Qiagen, Inc. Hilden, Germany) and TRANSFECTAM (Promega Biotec, Inc., Madison, WI), as well as other liposomes developed according to procedures standard in the art. In addition, the nucleic acid or vector of this invention can be delivered *in vivo* by electroporation, the technology for which is available from Genetronics, Inc. (San Diego, CA) as well as by means of a SONOPORATION machine (ImaRx Pharmaceutical Corp.,
30 Tucson, AZ).

173. The materials may be in solution, suspension (for example, incorporated into microparticles, liposomes, or cells). These may be targeted to a particular cell type via antibodies, receptors, or receptor ligands. These techniques can be used for a variety of

other specific cell types. Vehicles such as "stealth" and other antibody conjugated liposomes (including lipid mediated drug targeting to colonic carcinoma), receptor mediated targeting of DNA through cell specific ligands, lymphocyte directed tumor targeting, and highly specific therapeutic retroviral targeting of murine glioma cells *in vivo*. The

5 following references are examples of the use of this technology to target specific proteins to tumor tissue. In general, receptors are involved in pathways of endocytosis, either constitutive or ligand induced. These receptors cluster in clathrin-coated pits, enter the cell via clathrin-coated vesicles, pass through an acidified endosome in which the receptors are sorted, and then either recycle to the cell surface, become stored intracellularly, or are
10 degraded in lysosomes. The internalization pathways serve a variety of functions, such as nutrient uptake, removal of activated proteins, clearance of macromolecules, opportunistic entry of viruses and toxins, dissociation and degradation of ligand, and receptor-level regulation. Many receptors follow more than one intracellular pathway, depending on the cell type, receptor concentration, type of ligand, ligand valency, and ligand concentration.

15 174. Nucleic acids that are delivered to cells which are to be integrated into the host cell genome, typically contain integration sequences. These sequences are often viral related sequences, particularly when viral based systems are used. These viral integration systems can also be incorporated into nucleic acids which are to be delivered using a non-nucleic acid based system of delivery, such as a liposome, so that the nucleic acid contained
20 in the delivery system can be come integrated into the host genome.

175. Other general techniques for integration into the host genome include, for example, systems designed to promote homologous recombination with the host genome. These systems typically rely on sequence flanking the nucleic acid to be expressed that has enough homology with a target sequence within the host cell genome that recombination
25 between the vector nucleic acid and the target nucleic acid takes place, causing the delivered nucleic acid to be integrated into the host genome. These systems and the methods necessary to promote homologous recombination are known to those of skill in the art.

176. As described above, the compositions can be administered in a
30 pharmaceutically acceptable carrier and can be delivered to the subject's cells *in vivo* and/or *ex vivo* by a variety of mechanisms well known in the art (e.g., uptake of naked DNA, liposome fusion, intramuscular injection of DNA via a gene gun, endocytosis and the like).

177. If *ex vivo* methods are employed, cells or tissues can be removed and maintained outside the body according to standard protocols well known in the art. The compositions can be introduced into the cells via any gene transfer mechanism, such as, for example, calcium phosphate mediated gene delivery, electroporation, microinjection or
5 proteoliposomes. The transduced cells can then be infused (e.g., in a pharmaceutically acceptable carrier) or homotopically transplanted back into the subject per standard methods for the cell or tissue type. Standard methods are known for transplantation or infusion of various cells into a subject.

178. The nucleic acids that are delivered to cells typically contain expression
10 controlling systems. For example, the inserted genes in viral and retroviral systems usually contain promoters, and/or enhancers to help control the expression of the desired gene product. A promoter is generally a sequence or sequences of DNA that function when in a relatively fixed location in regard to the transcription start site. A promoter contains core elements required for basic interaction of RNA polymerase and transcription factors, and
15 may contain upstream elements and response elements.

179. Preferred promoters controlling transcription from vectors in mammalian host cells may be obtained from various sources, for example, the genomes of viruses such as polyoma, Simian Virus 40 (SV40), adenovirus, retroviruses, hepatitis-B virus and most preferably cytomegalovirus, or from heterologous mammalian promoters, e.g. beta actin
20 promoter. The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment which also contains the SV40 viral origin of replication. The immediate early promoter of the human cytomegalovirus is conveniently obtained as a *HindIII* E restriction fragment. Of course, promoters from the host cell or related species also are useful herein.

25 180. Enhancer generally refers to a sequence of DNA that functions at no fixed distance from the transcription start site and can be either 5' to the transcription unit. Furthermore, enhancers can be within an intron (Banerji, J.L. et al., *Cell* 33: 729 (1983)) as well as within the coding sequence itself (Osborne, T.F., et al., *Mol. Cell Bio.* 4: 1293 (1984)). They are usually between 10 and 300 bp in length, and they function in cis.
30 Enhancers function to increase transcription from nearby promoters. Enhancers also often contain response elements that mediate the regulation of transcription. Promoters can also contain response elements that mediate the regulation of transcription. Enhancers often determine the regulation of expression of a gene. While many enhancer sequences are now

known from mammalian genes (globin, elastase, albumin, α -fetoprotein and insulin), typically one will use an enhancer from a eukaryotic cell virus for general expression. Preferred examples are the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late
5 side of the replication origin, and adenovirus enhancers.

181. The promotor and/or enhancer may be specifically activated either by light or specific chemical events which trigger their function. Systems can be regulated by reagents such as tetracycline and dexamethasone. There are also ways to enhance viral vector gene expression by exposure to irradiation, such as gamma irradiation, or alkylating
10 chemotherapy drugs.

182. In certain embodiments the promoter and/or enhancer region can act as a constitutive promoter and/or enhancer to maximize expression of the region of the transcription unit to be transcribed. In certain constructs the promoter and/or enhancer region be active in all eukaryotic cell types, even if it is only expressed in a particular type
15 of cell at a particular time. A preferred promoter of this type is the CMV promoter (650 bases). Other preferred promoters are SV40 promoters, cytomegalovirus (full length promoter), and retroviral vector LTR.

183. It has been shown that all specific regulatory elements can be cloned and used to construct expression vectors that are selectively expressed in specific cell types such as
20 melanoma cells. The glial fibrillary acidic protein (GFAP) promoter has been used to selectively express genes in cells of glial origin.

184. Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human or nucleated cells) may also contain sequences necessary for the termination of transcription that may affect mRNA expression. These regions are transcribed as
25 polyadenylated segments in the untranslated portion of the mRNA encoding tissue factor protein. The 3' untranslated regions also include transcription termination sites. It is preferred that the transcription unit also contain a polyadenylation region. One benefit of this region is that it increases the likelihood that the transcribed unit will be processed and transported like mRNA. The identification and use of polyadenylation signals in
30 expression constructs is well established. It is preferred that homologous polyadenylation signals be used in the transgene constructs. In certain transcription units, the polyadenylation region is derived from the SV40 early polyadenylation signal and consists of about 400 bases. It is also preferred that the transcribed units contain other standard

sequences alone or in combination with the above sequences improve expression from, or stability of, the construct.

185. The viral vectors can include a nucleic acid sequence encoding a marker product. This marker product is used to determine if the gene has been delivered to the cell and once delivered is being expressed. Preferred marker genes are the *E. Coli* lacZ gene, which encodes β -galactosidase, and green fluorescent protein.

186. In some embodiments the marker may be a selectable marker. Examples of suitable selectable markers for mammalian cells are dihydrofolate reductase (DHFR), thymidine kinase, neomycin, neomycin analog G418, hygromycin, and puromycin. When such selectable markers are successfully transferred into a mammalian host cell, the transformed mammalian host cell can survive if placed under selective pressure. There are two widely used distinct categories of selective regimes. The first category is based on a cell's metabolism and the use of a mutant cell line which lacks the ability to grow independent of a supplemented media. Two examples are: CHO DHFR- cells and mouse LTK- cells. These cells lack the ability to grow without the addition of such nutrients as thymidine or hypoxanthine. Because these cells lack certain genes necessary for a complete nucleotide synthesis pathway, they cannot survive unless the missing nucleotides are provided in a supplemented media. An alternative to supplementing the media is to introduce an intact DHFR or TK gene into cells lacking the respective genes, thus altering their growth requirements. Individual cells that were not transformed with the DHFR or TK gene will not be capable of survival in non-supplemented media.

187. The second category is dominant selection which refers to a selection scheme used in any cell type and does not require the use of a mutant cell line. These schemes typically use a drug to arrest growth of a host cell. Those cells that have a novel gene would express a protein conveying drug resistance and would survive the selection. Examples of such dominant selection use the drugs neomycin, mycophenolic acid, or hygromycin,. The three examples employ bacterial genes under eukaryotic control to convey resistance to the appropriate drug G418 or neomycin (geneticin), xgpt (mycophenolic acid) or hygromycin, respectively. Others include the neomycin analog G418 and puramycin.

188. Disclosed herein are kits that are drawn to reagents that can be used in practicing the methods disclosed herein. The kits can include any reagent or combination of reagents discussed herein or that would be understood to be required or beneficial in the

practice of the disclosed methods. For example, the kits could include primers to perform the amplification reactions discussed in certain embodiments of the methods, as well as the buffers and enzymes required to use the primers as intended. The kits could include systems comprising the essential elements of SMD activity.

189. The compositions disclosed herein and the compositions necessary to perform the disclosed methods can be made using any method known to those of skill in the art for that particular reagent or compound unless otherwise specifically noted.

190. The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how the compounds, compositions, articles, devices and/or methods claimed herein are made and evaluated, and are intended to be purely exemplary of the invention and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.), but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C or is at ambient temperature, and pressure is at or near atmospheric.

Example 1: Identification of mRNAs bound by Staufen-1

Table 3. Genes that Encode Putative Stau1-binding mRNAs as Determined by Microarray Analysis

Gene Symbol	Accession Number	Unigene Name
ARF1	AA580004	ADP-ribosylation factor 1
MGC14799	BC005995	hypothetical protein MGC14799
GNAS	AF064092	GNAS complex locus
DFFA	NM_004401	DNA fragmentation factor 45kDa alpha polypeptide
CSDA	NM_003651	cold shock domain protein A
SEC61A1	NM_013336	Sec61 alpha 1 subunit (<i>S. cerevisiae</i>)
PSMD12	NM_002816	proteasome (prosome macropain) 26S subunit non-ATPase 12
EIF5A	NM_001970	eukaryotic translation initiation factor 5A
C4orf9	R06783	chromosome 4 open reading frame 9
FLJ10613	NM_019067	hypothetical protein FLJ10613
GNE	NM_005476	glucosamine (UDP-N-acetyl)-2-epimerase/N-acetylmannosamine kinase
FLJ30656	AW873564	<i>H. sapiens</i> transcribed sequences
LOC149603	AA085748	hypothetical protein LOC149603
TEGT	NM_003217	testis enhanced gene transcript (BAX inhibitor 1)
MCM4	AI859865	MCM4 minichromosome maintenance deficient 4 (<i>S. cerevisiae</i>)
LOC63929	NM_022098	hypothetical protein LOC63929
KIAA0186	NM_021067	KIAA0186 gene product
PRKAR2A	BF246917	protein kinase cAMP-dependent regulatory type II alpha
NUTF2	NM_005796	nuclear transport factor 2
GDF1	NM_001492	growth differentiation factor 1
PAICS	AA902652	phosphoribosylaminoimidazole carboxylase
		phosphoribosylaminoimidazole succinocarboxamide synthetase
TMPO	AF113682	thymopoietin
1274107		

AAMP NM_001087 angio-associated migratory cell protein

- 293 cells were transiently transfected with a plasmid that expressed Stau1-HA₃ or, to control for nonspecific IP, Stau1-6xHis. Biotin-labeled cRNA was synthesized from RNA that had been
 5 immunopurified using anti-HA antibody and hybridized to Affymetrix U133A microarrays. Changes of at least 2.5-fold were scored as Stau1-interacting transcripts.

Table 4. Transcripts up-regulated after down-regulating Stau1 in two independently performed analyses

(Note : Redundancies reflect different wells in each microarray)

AFFX-M27830_5_at	Unknown transcript
211506_s_at	Unknown transcript (don't know if same as above)
205660_at	2'-5'-oligoadenylate synthetase-like
210797_s_at	2'-5'-oligoadenylate synthetase-like /// 2'-5'-oligoadenylate synthetase-like
212543_at	absent in melanoma 1
200974_at	actin alpha 2 smooth muscle aorta
204470_at	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity alpha)
228335_at	claudin 11 (oligodendrocyte transmembrane protein)
202712_s_at	creatine kinase mitochondrial 1 (ubiquitous)
219863_at	cyclin-E binding protein 1
210764_s_at	cysteine-rich angiogenic inducer 61
232165_at	epiplakin 1
232164_s_at	epiplakin 1
216442_x_at	fibronectin 1
210495_x_at	fibronectin 1
212464_s_at	fibronectin 1
211719_x_at	fibronectin 1 /// fibronectin 1
212473_s_at	flavoprotein oxidoreductase MICAL2
226269_at	ganglioside-induced differentiation-associated protein 1
204471_at	growth associated protein 43
205184_at	guanine nucleotide binding protein (G protein) gamma 4
209657_s_at	heat shock transcription factor 2
227547_at	Homo sapiens transcribed sequence with moderate similarity to protein ref:NP_071431.1 (H.sapiens) cytokine receptor-like factor 2; cytokine receptor CRL2 precursor [Homo sapiens]
230831_at	Homo sapiens transcribed sequences
218986_s_at	hypothetical protein FLJ20035
235417_at	hypothetical protein FLJ25348
1562415_a_at	hypothetical protein FLJ25348
212909_at	hypothetical protein MGC29643
211959_at	insulin-like growth factor binding protein 5
214453_s_at	interferon-induced protein 44
226757_at	interferon-induced protein with tetratricopeptide repeats 2
229450_at	interferon-induced protein with tetratricopeptide repeats 4
205798_at	interleukin 7 receptor
202859_x_at	interleukin 8
201650_at	keratin 19 /// keratin 19
224657_at	mitogen-inducible gene 6
242456_at	MRE11 meiotic recombination 11 homolog A (S. cerevisiae)

210809_s_at	osteoblast specific factor 2 (fasciclin I-like)
201288_at	Rho GDP dissociation inhibitor (GDI) beta
204035_at	secretogranin II (chromogranin C)
59705_at	selenocysteine lyase
222557_at	stathmin-like 3
221477_s_at	superoxide dismutase 2 mitochondrial
220325_at	TAF7-like RNA polymerase II TATA box binding protein (TBP)- associated factor 50kDa
235086_at	thrombospondin 1
201108_s_at	thrombospondin 1
201109_s_at	thrombospondin 1
209277_at	tissue factor pathway inhibitor 2
205547_s_at	transgelin
1555724_s_at	transgelin
206508_at	tumor necrosis factor (ligand) superfamily member 7
202330_s_at	uracil-DNA glycosylase

191. The microarray results indicate that SMD can be utilized by mammalian cells to regulate the abundance of hundreds of cellular transcripts and, hence, expression of the encoded proteins. Transcripts identified to be regulated by SMD have a broad range of cellular functions that include signal transduction, cell proliferation, cell metabolism, immune response, DNA repair, and transcriptional regulation. Therefore, SMD can play a key role in establishing and maintaining cellular homeostasis. For example, SMD naturally targets transcripts encoding the IL-7 receptor (IL-7R), c-JUN, and SERPINE1 (also called PAI1). Down regulating cellular Stau1 or Upf1 but not Upf2 increased the abundance of each cellular mRNA.

Example 2: A Minimized Stau1 Binding Site Resides $\geq$ 67 Nucleotides Downstream of the Normal Termination Codon of Arf1 mRNA and Mediates mRNA Decay

192. The finding in two independently performed microarray analyses that there are at least 23 293-cell mRNAs that bind Stau1 (Table 3) indicates that SMD is used by cells to coordinately regulate a battery of genes – a number of which are involved in cell growth, division or both – in response to changes in the cellular abundance or specific activity of Stau1, Upf1 or both (see below). If binding is sufficiently downstream of the normal termination codon, then these mRNAs should, like Arf1 mRNA, be natural targets of SMD in a mechanism that is EJC-independent.

193. To construct pcFLuc(UAA $\rightarrow$ CAA)-MS2bs, which lacks a termination codon upstream of the MS2 binding sites, pcFLuc-8bs that had been digested with NotI and EcoRV was ligated to a PCR-amplified fragment that contains C-terminus of FLuc in which the UAA codon was converted to a CAA codon and had been digested with NotI and EcoRV. The PCR

reactions were performed using pR/HCV/F and two primers: 5'-TTGACCGCTTGAAGTCTTTAATTAAATAC-3' (sense) (SEQ ID NO: 34) and 5'-CGAAGCGGCCGCAATTACATTTTGAATTTGGACTTCCGCCCTTCTTGGC-3' (antisense) (SEQ ID NO: 35). Underlined nucleotides specify a NotI site.

5 Example 3: Identification of HeLa-cell transcripts are regulated upon Stau1 depletion

194. To identify physiologic SMD targets, HeLa-cell RNA from three independently performed transfections, in which the level of cellular Stau1 was depleted to as little as 4% of normal (where normal is defined as the level in the presence of Control siRNA), was separately hybridized to microarrays. Sequences from 18,279 HeLa-cell transcripts were analyzed, representing 34% of the array probe sets, in all three hybridization experiments. It was observed that 124 transcripts, or 1.1 % of the HeLa-cell transcriptome that was analyzed, were upregulated at least 2-fold in all three transfections (Table 5).

Table 5: Transcripts upregulated in human cells depleted of Stau1 in three independently performed microarray analyses

<i>Transcript</i>	<i>Fold change</i>	<i>Abbreviation</i>	<i>Probe set</i>
<i>Homo sapiens</i> hypothetical protein LOC339468 mRNA (cDNA clone IMAGE:5166507) partial cds	9.79		1562908_at
I factor (complement)	8.78	IF	1555564_a_at
fibronectin 1	7.98	FN1	216442_x_at
interferon-induced protein with tetratricopeptide repeats 2	7.51	IFIT2	226757_at
	5.91		211506_s_at
interferon-induced protein 44	5.88	IFI44	214059_at
secretogranin II (chromogranin C)	5.62	SCG2	204035_at
stathmin-like 3	5.53	STMN3	222557_at
growth associated protein 43	5.48	GAP43	204471_at
creatine kinase mitochondrial 1 (ubiquitous)	5.35	CKMT1	202712_s_at
osteoblast specific factor 2 (fascin I-like)	5.30	OSF-2	210809_s_at
hypothetical protein FLJ25348	4.77	FLJ25348	1562415_a_at
guanine nucleotide binding protein (G protein) gamma 4	4.75	GNG4	205184_at
hypothetical protein FLJ33505	4.73	FLJ33505	1561114_a_at
filamin-binding LIM protein-1	4.71	FBLP-1	1555480_a_at
claudin 11 (oligodendrocyte transmembrane protein)	4.50	CLDN11	228335_at
<i>Homo sapiens</i> transcribed sequence with weak similarity to protein ref:NP_060265.1 (<i>H.sapiens</i>) hypothetical protein FLJ20378 [<i>Homo sapiens</i>]	4.46		235629_at
insulin-like growth factor binding protein 5	4.30	IGFBP5	211959_at
integrin beta 3 (platelet glycoprotein IIIa antigen CD61)	4.18	ITGB3	204627_s_at
interferon-induced protein with tetratricopeptide repeats 4	4.13	IFIT4	229450_at
alpha-actinin-2-associated LIM protein	3.97	ALP	210170_at
transgelin	3.94	TAGLN	205547_s_at
2'-5'-oligoadenylate synthetase-like /// 2'-5'-oligoadenylate synthetase-like	3.86	OASL	210797_s_at
tissue factor pathway inhibitor 2	3.82	TFPI2	209277_at
catenin (cadherin-associated protein) alpha-like 1	3.80	CTNNAL1	213712_at
<i>Homo sapiens</i> cDNA: FLJ20914 fis clone ADSE00646	3.76		234597_at
thrombospondin 1	3.73	THBS1	201108_s_at
protein tyrosine phosphatase receptor type O	3.62	PTPRO	1554199_at
cyclin-E binding protein 1	3.60	CEB1	219863_at

NADPH oxidase 4	3.59	NOX4	219773_at
hypothetical protein MGC29643	3.58	MGC29643	212909_at
<i>Homo sapiens</i> uncharacterized gastric protein ZA43P mRNA partial cds	3.43		232696_at
<i>Homo sapiens</i> similar to KIAA0563-related gene (LOC376854) mRNA	3.42		1562921_at
hypothetical protein FLJ20035	3.40	FLJ20035	218986_s_at
<i>Homo sapiens</i> cDNA FLJ41180 fis clone BRACE2043142	3.40		227890_at
tissue factor pathway inhibitor 2	3.35	TFPI2	209278_s_at
absent in melanoma 1	3.34	AIM1	212543_at
chromosome 14 open reading frame 141	3.29	C14orf141	223690_at
dual specificity phosphatase 6	3.29	DUSP6	208891_at
interferon alpha-inducible protein (clone IFI-15K)	3.19	G1P2	205483_s_at
serine (or cysteine) proteinase inhibitor clade E (nexin plasminogen activator inhibitor type 1) member 1	3.16	SERPINE1	202628_s_at
<i>Homo sapiens</i> cDNA FLJ23692 fis clone HEP10227	3.13		235846_at
hypothetical protein FLJ20637	3.04	FLJ20637	219352_at
signaling lymphocytic activation molecule family member 1	3.00	SLAMF1	206181_at
selenocysteine lyase	2.98	SCLY	59705_at
interleukin 7 receptor	2.97	IL7R	205798_at
Rho GDP dissociation inhibitor (GDI) beta	2.94	ARHGDI B	201288_at
actin alpha 2 smooth muscle aorta	2.94	ACTA2	200974_at
chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity alpha)	2.84	CXCL1	204470_at
DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide	2.83	RIG-I	218943_s_at
insulin-like growth factor binding protein 5	2.78	IGFBP5	211958_at
interferon-induced protein with tetratricopeptide repeats 1	2.78	IFIT1	203153_at
<i>Homo sapiens</i> hypothetical protein LOC285103 mRNA (cDNA clone IMAGE:5273139) partial cds	2.76		227966_s_at
decapping enzyme hDcp2	2.74	DCP2	212919_at
hypothetical protein FLJ34064	2.73	FLJ34064	1553244_at
eukaryotic translation initiation factor 5A2	2.73	EIF5A2	235289_at
hypothetical protein MGC19764	2.71	MGC19764	1557078_at
<i>Homo sapiens</i> BIC noncoding mRNA complete sequence transducin-like enhancer of split 4 (E(sp1) homolog <i>Drosophila</i>)	2.69		229437_at
dapper homolog 1 antagonist of beta-catenin (xenopus)	2.68	TLE4	235765_at
chromosome 9 open reading frame 39	2.67	DACT1	219179_at
<i>Homo sapiens</i> transcribed sequences	2.64	C9orf39	220095_at
v-jun sarcoma virus 17 oncogene homolog (avian)	2.61		229242_at
integrin alpha 4 (antigen CD49D alpha 4 subunit of VLA-4 receptor)	2.58	JUN	201466_s_at
<i>Homo sapiens</i> cDNA FLJ40697 fis clone THYMU2025406	2.56	ITGA4	205885_s_at
<i>Homo sapiens</i> transcribed sequences	2.53		235203_at
tenascin C (hexabrachion)	2.53		236817_at
hypothetical protein FLJ13621	2.52	TNC	201645_at
flavoprotein oxidoreductase MICAL2	2.50	FLJ13621	207286_at
<i>Homo sapiens</i> cDNA FLJ46457 fis clone THYMU3020856	2.47	MICAL2	212473_s_at
thymosin beta identified in neuroblastoma cells	2.44		225007_at
prostaglandin E receptor 4 (subtype EP4)	2.44	TMSNB	205347_s_at
chromosome 14 open reading frame 128	2.42	PTGER4	204897_at
ganglioside-induced differentiation-associated protein 1	2.41	C14orf128	228889_at
zinc finger protein 36 C3H type-like 1	2.41	GDAP1	226269_at
uracil-DNA glycosylase	2.38	ZFP36L1	211965_at
hypothetical protein MGC27277	2.37	UNG	202330_s_at
<i>Homo sapiens</i> cDNA FLJ10158 fis clone HEMBA1003463.	2.37	MGC27277	242283_at
CDK5 regulatory subunit associated protein 1-like 1	2.35		232125_at
	2.34	CDKAL1	214877_at

<i>Homo sapiens</i> transcribed sequence with moderate similarity to protein ref:NP_071431.1 (<i>H.sapiens</i>) cytokine receptor-like factor 2	2.32		227547_at
aldehyde dehydrogenase 1 family member A3	2.32	ALDH1A3	203180_at
proteasome (prosome macropain) subunit beta type 9 (large multifunctional protease 2)	2.29	PSMB9	204279_at
PHD finger protein 11	2.27	PHF11	221816_s_at
B-cell scaffold protein with ankyrin repeats 1	2.27	BANK1	219667_s_at
KIAA0143 protein	2.26	KIAA0143	212150_at
guanylate binding protein 1 interferon-inducible 67kDa	2.26	GBP1	202270_at
epiplakin 1	2.25	EPPK1	232164_s_at
actinin alpha 1	2.24	ACTN1	211160_x_at
ring finger protein 20	2.22	RNF20	222683_at
leucine rich repeat (in FLII) interacting protein 1	2.22	LRRFIP1	223492_s_at
<i>Homo sapiens</i> cDNA FLJ39819 fis clone SPLEN2010534.	2.21		1556111_s_at
hypothetical protein LOC150759	2.21	LOC150759	213703_at
dihydropyrimidinase-like 3	2.20	DPYSL3	201431_s_at
type I transmembrane receptor (seizure-related protein)	2.20	PSK-1	233337_s_at
zinc finger protein 90 homolog (mouse)	2.20	ZFP90	235698_at
tumor protein p53 (Li-Fraumeni syndrome)	2.20	TP53	211300_s_at
<i>Homo sapiens</i> cDNA FLJ11465 fis clone HEMBA1001636.	2.19		228632_at
dnaj-like protein	2.17	LOC148418	229402_at
transforming growth factor beta 1 induced transcript 1	2.15	TGFB11	209651_at
ets variant gene 1	2.15	ETV1	221911_at
Rho GTPase activating protein 19	2.14	ARHGAP19	212738_at
<i>Homo sapiens</i> hypothetical LOC284120 (LOC284120) mRNA	2.13		241394_at
suppressor of cytokine signaling 2	2.13	SOCS2	203373_at
cyclin-dependent kinase 5 regulatory subunit 1 (p35)	2.11	CDK5R1	204995_at
likely ortholog of mouse Sds3	2.11	SDS3	233841_s_at
<i>Homo sapiens</i> transcribed sequences	2.09		244091_at
spinal cord-derived growth factor-B	2.09	SCDGF-B	219304_s_at
<i>Homo sapiens</i> cDNA FLJ37290 fis clone BRAMY2014469.	2.09		231026_at
histone deacetylase 8	2.07	HDAC8	223908_at
	2.06		1567079_at
<i>Homo sapiens</i> transcribed sequence with weak similarity to protein pir:138588 (<i>H.sapiens</i>) 138588 reverse transcriptase homolog - human retrotransposon L1	2.06		235302_at
<i>Homo sapiens</i> full length insert cDNA clone YP61C10	2.06		1561631_at
high-mobility group box 3	2.05	HMGB3	225601_at
KIAA0931 protein	2.05	KIAA0931	213407_at
<i>Homo sapiens</i> cDNA FLJ33441 fis clone BRACE2021932.	2.04		1556081_at
hypothetical protein DKFZp761K1423	2.04	DKFZp761K1423	218613_at
protein tyrosine phosphatase receptor type F	2.04	PTPRF	200635_s_at
thioredoxin reductase 3	2.03	TXNRD3	59631_at
CGI-72 protein	2.03	CGI-72	231967_at
hypothetical protein MGC15634	2.03	MGC15634	242923_at
enoyl-Coenzyme A hydratase/3-hydroxyacyl Coenzyme A dehydrogenase	2.03	EHHADH	205222_at
hairy and enhancer of split 1 (<i>Drosophila</i>)	2.02	HES1	203394_s_at
chromosome 11 open reading frame 9	2.00	C11orf9	204073_s_at
TIA1 cytotoxic granule-associated RNA binding protein	2.00	TIA1	1554890_a_at

195. The validity of the microarray results was tested for 12 of the upregulated transcripts and 6 of the downregulated transcripts using RT-PCR and a primer pair that is specific for each transcript (Table 6).

Table 6 Primer pairs used to amplify transcripts found in microarray analyses to be upregulated or downregulated in human cells

Gene	Primer (Sense)	Primer (Antisense)
c-JUN	5'-CTT GAA AGC TCA GAA CTC GG-3'	5'-TCA GCC CCC GAC GGT CTC TC-3'
SERPINE1	5'-ACC GCC AAT CGC AAG GCA CC-3'	5'-GCT GAT CTC ATC CTT GTT CC-3'
IL7R	5'-AAG TGG CTA TGC TCA AAA TG-3'	5'-TTC AGG CAC TTT ACC TCC AC-3'
IF	5'-CCT TGA CCT TGG GTT TCA AC-3'	5'-ATT TGC AAT GGA AGC CTT TG-3'
GAP43	5'-TGT GCT GTA TGA GAA GAA CC-3'	5'-GCT TCA TCC TTC TTA TTA GC-3'
CKMT1	5'-GAC TGG CCA GAT GCT CGT GG-3'	5'-ATC TTT GGG AAG CGG CTA TC-3'
STMN3	5'-CCA TGG CCA GCA CCA TTT CC-3'	5'-ACC TCG GCC GCG TGC AGC TC-3'
TAGLN	5'-TCC TTC CTG CGA GCC CTG AG-3'	5'-GCA CTG CTG CCA TGT CTT TG-3'
OASL	5'-TGC AAT CAT TGA GGA TTG TG-3'	5'-CAC TGT CAA GTG GAT GTC TC-3'
GDI1	5'-GAC AGA GAC GTG AAG CAC TG-3'	5'-CCA TAA ATG TTG CTT TAT CC-3'
CXCL1	5'-CCT GGT AGC CGC TGG CCG GC-3'	5'-CTT CTG GTC AGT TGG ATT TG-3'
DCP2	5'-GAT TTA TGT TGT TGT AGT TG-3'	5'-CCA AGC AGC CAA TTT TAT TG-3'
TMSL8	5'-ACA GCC TTT CAC GAG TCT TC-3'	5'-CTG CTG TTG GGA GGC GAT CC-3'
PSMB9	5'-GCG GGA GAA GTC CAC ACC GG-3'	5'-AGG CTG TCG AGT CAG CAT TC-3'
GDAP1	5'-AGT TAA CTG TGG ACT CCA TG-3'	5'-ACT TTC TCC AAC TCA TCA AG-3'

Results demonstrated that 11 of the 12 were increased in abundance by 1.5-fold to 8.5-fold (Figure 1) upon Stau1 depletion. Therefore, the microarray results can generally be viewed as a reliable assessment of changes in transcript abundance upon Stau1 depletion.

Example 4: Stau1 or Upf1 depletion increases the abundance of c-JUN, SERPINE and IL7R 3' mRNAs

196. Four of the transcripts that were found to be upregulated when Stau1 is depleted were also found in microarray analyses to be upregulated when Upf1 is depleted (Table 7).

Table 7 Transcripts upregulated in human cells depleted of either Stau1 (three independently performed microarray analyses in this study) or Upf1 (Mendell et al., 2004)

Transcript	Product function	Relative increase (microarray value)	
		Stau1 depletion	Upf1 depletion
Serine (or cysteine) proteinase inhibitor clade E	Bait for tissue plasminogen activator	3.2	3.8

(nexin plasminogen activator inhibitor type 1) member 1 (SERPINE1)	urokinase, and protein C		
Interleukin 7 receptor (IL7R)	Receptor for interleukin 7	3.0	2.1
v-jun sarcoma virus 17 oncogene homolog (avian) (c-JUN)	Proto oncogene	2.6	5.3
Protein tyrosine phosphatase receptor type F (PTPRF)	Cell adhesion receptor	2.0	2.8

Interestingly, the upregulation of three of these transcripts could not be explained by the EJC-dependent rule that applies to NMD. The three transcripts encode serine (or cysteine) proteinase inhibitor clade E (nexin plasminogen activator inhibitor type 1) member 1 (Serpine1), interleukin 7 receptor (IL7R), and v-jun sarcoma virus 17 oncogene homolog (avian) (c-jun).

197. To assess the possibility that each transcript is an SMD target, HeLa cells were transiently transfected with one of six small interfering (si) RNAs (Kim et al., 2005): Stau1 or Stau1(A) siRNA, which targeted a different Stau1 mRNA sequence; Upf1 or Upf1(A) siRNA, which targeted a different UPF1 mRNA sequence; Upf2 siRNA, which has no effect on SMD; or a nonspecific Control siRNA. Two days later, protein and RNA were isolated for analysis using Western blotting and RT-PCR, respectively.

198. Western blotting revealed that Stau1 or Stau1(A) siRNA depleted the cellular level of Stau1 to 21% or 3% of normal, respectively, Upf1 or Upf1(A) siRNA depleted the cellular the level of Upf1 to 1% or 2% of normal, respectively, and Upf2 siRNA depleted the cellular level of Upf2 to 1% of normal (Figure 2A, where normal in each case is defined as the level in the presence of Control siRNA after normalization to the level of Vimentin). It was found that c-JUN, SERPINE and IL7R transcripts were upregulated 2.1-fold to 3.8-fold when Stau1 or Upf1 was depleted, but unaffected when Upf2 was depleted (Figure 2B, where each transcript is normalized to the level of SMG7 mRNA). These results indicate that each transcript is targeted for SMD.

Example 5: Stau1 binds the 3' UTR of c-JUN, SERPINE and IL7R 3' mRNAs

199. To further investigate whether each of the three transcripts is an SMD target, (i) nucleotides 481-671 of the c-JUN 3' UTR, which include the 151-nucleotide class III (i.e., non-AUUUA) AU-rich element (ARE)(Peng et al., 1996) plus 45 flanking nucleotides; (ii) nucleotides 1-1575 of the SERPINE1 3' UTR or (iii) nucleotides 1-339 of IL7R mRNA were inserted immediately downstream of the Firefly (F) Luciferase (Luc) translation termination codon within pcFLuc. For each 3' UTR, nucleotide 1 is defined as the nucleotide immediately

3' to the normal termination codon. The encoded hybrid transcripts were tested for Stau1-HA₃ binding.

Example 6: lncRNAs transactivate SMD by duplexing with 3' UTRs via Alu elements

200. The failure using Mfold to identify dsRNA structures similar to the SBS of ARF1 mRNA within the 3'UTRs of other SMD targets led to the observation that two well characterized SMD targets – plasminogen activator inhibitor type 1 (SERPINE1) mRNA and hypothetical protein FLJ21870 mRNA – contain a single 3'UTR Alu element. Approximately 13% of the ~1.6% of protein-encoding transcripts in human epithelial HeLa cells that are upregulated at least 1.8-fold upon STAU1 downregulation in three independently performed microarray analyses contain a single 3'UTR Alu element (Table 8). This percentage is higher than the ~4% of HeLa-cell protein encoding transcripts that contain one or more 3'UTR Alu elements, indicating that 3'UTR Alu elements are enriched in SMD targets relative to the bulk of cellular mRNAs.

Table 8. Human genes encoding transcripts that are upregulated at least 1.8-fold upon STAU1 downregulation and contain a single 3'UTR Alu element

Gene symbol	NCBI accession #	Fold upregulation *	Alu motif in 3'UTR	Full name of gene
IFI44	NM_006417	5.88	AluSq	interferon-induced protein 44
FBLIM1	NM_017556	4.71	AluSz	filamin-binding LIM protein 1
CLDN11	NM_005602	4.50	AluJb	claudin 11
PDLIM3	NM_014476	3.97	AluSx	PDZ and LIM domain 3
OASL	NM_003733	3.86	AluJo	2'-5'-oligoadenylate synthetase-like TAF7-like RNA polymerase II, TATA box-binding protein (TBP)-associated factor, 50kDa
TAF7L	NM_024885	3.72	FLAM_A	
NOX4	NM_016931	3.59	AluSg7	NADPH oxidase 4
AIM1	NM_001624	3.34	AluJb	absent in melanoma 1 serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1
SERPINE1	NM_000602	3.16	AluJo	DCP2 decapping enzyme homolog (S. cerevisiae)
DCP2	NM_152624	2.74	AluSp	eukaryotic translation initiation factor 5A2
EIF5A2	NM_020390	2.73	AluSx3	centrosomal protein 135kDa
CEP135	NM_025009	2.50	AluSx1	leucine-rich repeat (in FLII) interacting protein 1
LRRFIP1	NM_004735	2.22	AluSq2	zinc-finger protein 90 homolog (mouse)
ZFP90	NM_133458	2.20	AluJb	tumor protein p53
TP53	NM_000546	2.20	AluJb	PH domain and leucine-rich repeat protein phosphatase 2
PHLPP2	NM_015020	2.05	AluSx	enoyl-CoA, hydratase/3-hydroxyacyl CoA dehydrogenase
EHHADH	NM_001966	2.03	AluJr	coiled-coil domain-containing 125
CCDC125	NM_176816	1.99	AluY	CUB domain-containing protein 1
CDCP1	NM_022842	1.98	AluJb	NUAK family, SNF1-like kinase, 2
NUAK2	NM_030952	1.96	AluSp	

AKT2	NM_001626	1.84	AluJr	v-akt murine thymoma viral oncogene homolog 2
APPL1	NM_012096	1.83	AluY	adaptor protein, phosphotyrosine interaction, PH domain and leucine zipper-containing 1
COL16A1	NM_001856	1.81	FLAM_A	collagen, type XVI, alpha 1
RIPK1	NM_003804	1.81	AluJo	receptor (TNFRSF)-interacting serine-threonine kinase 1

*Minimum fold upregulation in each of three independently performed microarray analyses

201. Alu elements are the most prominent repeats in the human genome: they constitute more than 10% of DNA sequences, are present at up to 1.4 million copies per cell, and share a 300-nucleotide consensus sequence of appreciable similarity among subfamilies. To date, Alu elements have been documented to be *cis*-effectors of protein encoding gene expression by influencing transcription initiation or elongation, alternative splicing, A-to-I editing or translation initiation. Since ncRNAs that perfectly basepair with mRNA can function in *trans* to generate endogenous siRNAs, imperfect base-pairing between the Alu element of a ncRNA and the Alu element of an mRNA 3'UTR can create an SBS so as to regulate mRNA decay. The focus of the experiments disclosed herein were on mRNAs that contain a single 3'UTR Alu-element to avoid the possibility of intramolecular base-pairing between inverted Alu elements, which can result in A-to-I editing and nuclear retention.
202. Analysis of Antisense ncRNA Pipeline identified 378 lncRNAs that contain a single Alu element (Table 9).

Table 9. Features of lncRNAs that contain a single Alu element

lncRNA accession # *	lncRNA Alu motif **	ΔG (kcal/mol) of predicted duplexes formed between the Alu element of each specified lncRNA and the 3'UTR Alu element of four proven SMD targets ***			
		<u>SERPINE1</u> <u>mRNA</u>	<u>FLJ21870</u> <u>mRNA</u>	<u>CDCP1</u> <u>mRNA</u>	<u>MTAP mRNA</u>
ASO1026	FLAM_C	-48.5	-100.5	0	-100.5
ASO1113	AluSx	0	0	-44.2	-21.8
ASO1151	FLAM_C	0	-60.9	0	-38.7
ASO1162	AluSx	-37.8	-43.7	-12.8	-31.8
ASO1170	FRAM	0	-19.2	-91.3	0
ASO1178	FLAM_C	0	-60.7	0	-70.6
ASO1183	SVA_A	0	-83.2	-25.1	-24.7
ASO1195	FLAM_C	0	-81.4	-21.1	-65.1
ASO1208	SVA_F	-28.8	0	0	-45.6
ASO1213	SVA_F	-28.8	0	0	-45.6
ASO1220	AluY	-22.7	-90.5	-18.5	-83.9
ASO1259	AluJb	0	-25.7	-71.6	0
ASO1267	AluJb	-28.7	-21.2	-107.9	0
ASO1282	AluJb	0	0	-90.6	-19.9

ASO1285	AluSx	0	-61.4	0	-38.9
ASO1289	FLAM_C	0	0	-55.8	-23.4
ASO1296	AluJb	-12.5	-48.3	-9.7	-26.8
ASO1320	FLAM_C	-43	-28.4	0	-28.4
ASO1321	AluJb	-24.6	-54	0	-17.4
ASO1323	AluSx	0	-86.8	0	-42.1
ASO1326	AluJb	0	-29.5	-114.2	0
ASO1362	FLAM_C	0	-21.9	0	-23.9
ASO1379	FLAM_C	0	0	-40.1	0
ASO1392	AluJb	0	0	-107.9	0
ASO1409	AluJb	-41.8	-90.2	0	-46.9
ASO1410	AluJb	-41.8	-90.2	0	-46.9
ASO1433	FLAM_C	0	0	-26.4	0
ASO1445	tRNA-Ala-GCY_	0	-23	0	-27.9
ASO1446	AluSx	0	-85.2	0	-80.6
ASO1450	tRNA-Ala-GCY_	0	0	-32.9	0
ASO1456	FLAM_C	0	0	-34.8	0
ASO1470	AluSx	-18.8	0	0	-21.4
ASO1476	AluJb	-50	-106.8	-18.5	-107.4
ASO1480	FLAM_C	-13.5	-17.4	-96.7	0
ASO1483	AluSx	0	-68	-31.6	-66.4
ASO1487	AluJb	-37.1	-83.3	0	-83.2
ASO1488	FLAM_C	0	-25.5	-42.7	0
ASO1496	FLAM_C	0	0	-66.8	0
ASO1497	AluJb	0	-19.1	-69.8	-17.9
ASO1498	AluSx	0	-18.4	-103.8	-24.4
ASO1500	FLAM_C	0	-21.8	-66.3	-18.1
ASO1503	FLAM_C	0	-24.1	0	0
ASO1511	tRNA-Thr-ACA	0	0	0	0
ASO1514	AluJb	-49.6	-99.2	0	-94.3
ASO1521	tRNA-Ala-GCY_	0	0	0	-19.5
ASO1523	AluSx	0	-88.5	0	-43.8
ASO1535	AluJb	-48.5	-66.8	0	-66.3
ASO1544	AluJb	-48.1	-99.7	0	-96.4
ASO1546	AluJb	-48.3	-65.4	-24.9	-13.2
ASO1566	FLAM_C	0	-17	-47.4	-15.2
ASO1569	AluSx	0	-22.8	-71.5	-27.2
ASO1601	AluSx	0	-71	-21.1	-74.4
ASO1632	AluJb	-99.5	-222.1	-24.7	-84.2
ASO1636	AluJb	-84.8	-85.5	-9.7	-103.2
ASO1668	AluY	0	0	-112.8	0
ASO1674	AluSx	-35.7	-53.6	0	-116.7
ASO1700	AluY	-46	-122.6	-35.1	-125.6
ASO1705	FRAM	0	0	-81.2	-26.5
ASO1714	AluJb	0	0	-135.9	0
ASO1719	AluSx	0	-17.2	-148.9	-25.4
ASO1721	AluJb	-119.7	-99.2	-22.9	-77
ASO1726	AluSx	0	0	-118.6	0
ASO1729	AluSx	0	0	-107.3	-25.2
ASO1730	AluJb	0	0	-141.1	0
ASO1736	AluSx	-70.3	-258.5	-31.4	-230.1
ASO1737	AluY	-68.2	-339	-22.6	-235.5

ASO1751	AluSx	0	-39.2	-138.7	0
ASO1754	FRAM	-10.9	-29.3	-103.9	-14.9
ASO1757	FLAM_C	-110.8	-163.4	-18.1	-88.9
ASO1763	FLAM_C	0	0	-167.3	-23.4
ASO1766	AluJb	-134.1	-177.1	-16.3	-78.2
ASO1768	AluJb	0	0	-128.2	0
ASO1770	AluY	0	-26.7	-175.9	-32.5
ASO1781	AluSx	-61.9	-255.5	-36.7	-166
ASO1787	AluJb	-57.9	-236	-49.9	-104.8
ASO1794	FRAM	0	-52.8	-16.3	-71.9
ASO1803	AluSx	-16.5	0	-214.9	0
ASO1808	AluJb	0	-27.9	-133.9	-39.9
ASO1810	AluJb	0	-28.4	-189.3	-32.6
ASO1812	AluSx	-76.6	-226.2	0	-348.3
ASO1818	AluY	-62.2	-407.9	-23.6	-229.1
ASO1821	AluSx	-45.5	-108.9	-19.5	-126.2
ASO1823	AluJb	-63.7	-146.4	-26.6	-280.7
ASO1825	AluSx	-54.1	-118.7	-23.7	-120.8
ASO1827	AluSx	-123.4	-262.6	0	-260
ASO1829	FLAM_C	-95.9	-137.3	-22.3	-92.1
ASO1841	AluJb	0	-22.9	-99.6	-20.8
ASO1862	AluSx	0	0	-176.7	-33
ASO1871	FLAM_C	-115.9	-159.7	-12.2	-79.7
ASO1876	AluSx	-114	-203.4	-11.3	-197.5
ASO1880	AluJb	-55.4	-110.3	-16.6	-95.4
ASO1885	AluJb	-13.5	-16.3	-358.2	0
ASO1895	AluJb	-73.1	-157	-27	-113.5
ASO1897	AluSx	-70.1	-101.1	-18.5	-121.4
ASO1901	AluSx	-72.7	-204.9	-20.9	-341.3
ASO1906	AluSx	0	0	-193.9	-30.6
ASO1912	AluSx	-106.7	-445.6	-21.1	-255.7
ASO1928	AluJb	-88.5	-208	-8.3	-105.5
ASO1934	AluJb	0	0	-135.9	0
ASO1957	AluSx	-65.9	-232.4	0	-300
ASO1959	AluSx	0	-35.5	-181.4	-34
ASO1966	AluSx	-16.5	0	-214.9	0
ASO1980	AluSx	0	-27.5	-192.2	-30.6
ASO1993	AluSx	-26.6	-136.8	-38.8	-77.6
ASO1994	AluJb	-88.2	-207.7	-19.7	-105.5
ASO1995	AluSx	0	0	-118.6	0
ASO1998	AluSx	-108.2	-444.2	-27.6	-264.2
ASO2002	AluJb	-84.6	-302.6	0	-135.6
ASO2003	AluSx	0	0	-118.6	0
ASO2015	AluSx	0	0	-118.6	0
ASO2016	AluSx	-31.5	-49.3	0	-125.8
ASO2020	AluSx	0	0	-118.6	0
ASO2026	AluJb	-77.8	-113.2	-23.1	-99.1
ASO2028	AluSx	-125.2	-223.9	-34.2	-128.7
ASO2029	AluJb	-77.8	-113.2	-23.1	-99.1
ASO2031	AluSx	-13.9	0	-157.2	-13.1
ASO2037	AluSx	-76.9	-116.5	-22.4	-103.5
ASO2042	FLAM_C	0	0	-68.8	0
ASO2046	AluSx	0	0	-118.6	0
ASO2059	FRAM	-78	-73	-31.5	-65.2
ASO2066	AluJb	0	-21.7	-121.2	-25.9

ASO2070	AluSx	-114.2	-447.6	-19.7	-190.2
ASO2087	FLAM_C	-116.1	-163.8	-36.5	-87
ASO2100	FLAM_C	0	0	-153.3	0
ASO2101	AluSx	0	0	-179.1	-29.8
ASO2120	AluJb	0	0	-240.5	-21.7
ASO2124	FLAM_C	-89.4	-102.7	-37.4	-30.7
ASO2126	AluJb	-13.2	-36.1	-136	-39.8
ASO2127	AluSx	0	0	-210.2	-26.2
ASO2128	FLAM_C	-115.2	-155.5	0	-71.8
ASO2131	AluSx	0	0	-159.1	-16.3
	tRNA-Arg-				
ASO2147	AGA	-23.7	0	-19.3	-19.8
ASO2153	AluJb	0	-21.7	-121.2	-25.9
ASO2160	AluY	-62.1	-208.4	-18.8	-218.5
ASO2168	AluJb	0	-25.1	-144	-30.9
ASO2179	AluJb	0	-26.5	-202.3	0
ASO2181	AluY	-66.2	-210.7	-26.4	-286.2
ASO2185	AluSx	-70.8	-233.4	0	-360.9
ASO2186	AluSx	-69.1	-116	-15.4	-103.5
ASO2188	AluY	0	-23.6	-196.1	-29.5
ASO2192	AluSx	0	0	-118.6	0
ASO2209	AluSx	-96.3	-147.9	-39.9	-150.6
ASO2218	FRAM	-60.9	-79.6	-20.1	-114.9
ASO2236	AluSx	0	-20	-96.5	-33.9
ASO2239	FLAM_C	0	0	-114.3	-40.9
ASO2241	AluSx	0	-33.3	-193.9	-15.6
ASO2242	AluY	-27.9	-54.5	0	-90.9
ASO2262	AluJb	-116.5	-279.2	0	-105.5
ASO2282	AluY	0	-12.7	-165.3	-29.5
ASO2287	AluSx	0	0	-189.6	-12.6
ASO2299	AluJb	0	0	-78.4	0
ASO2301	AluJb	-42.8	-25.6	-20.4	-41
ASO2319	AluJb	-50.9	-60.1	0	-17.9
ASO2320	AluSx	0	-21.2	-79.8	-19.9
ASO2321	FLAM_C	-41.3	-53.5	0	-16.2
ASO2347	FLAM_C	-42.2	-79.9	0	-53.7
ASO2356	AluJb	0	0	-40.7	0
ASO2360	AluSx	0	-77.7	0	-29.3
ASO2362	AluJb	-27.9	-21.2	-98.8	-24.4
ASO2368	AluJb	-49.6	-57.5	0	-30.2
ASO2370	AluJb	-24.2	-12.3	-23.4	-31.8
ASO2373	AluJb	0	-14.4	0	0
ASO2378	AluSx	0	0	-28	0
ASO2385	AluSx	0	0	-46.7	0
ASO2395	FLAM_C	0	-60.5	-33.1	-23.2
ASO2414	FLAM_C	0	-68.3	0	-53
ASO2418	AluSx	0	-25.4	-85.4	-40.4
ASO2443	AluJb	-25.9	-41.8	-12.2	-36.7
ASO2469	AluJb	-61.3	-106.3	-39.2	-102.4
ASO2474	AluJb	0	0	-28.3	0
ASO2479	AluJb	-48.6	-111.1	-20.1	-94.5
ASO2488	AluJb	0	0	-39.3	0
	tRNA-Ala-				
ASO2490	GCY_	0	0	0	-21.7
ASO2495	AluJb	-53.4	-63.3	-22.2	-55.4

ASO2510	AluJb	-39	-72.6	-7.2	-48.6
ASO2515	AluJb	-49.6	-57.5	0	-30.2
ASO2518	AluJb	-57	-120.5	-11.7	-99.6
ASO2520	AluJb	0	0	-56.6	0
ASO2532	AluSx	0	-41.8	0	-24.8
ASO2565	AluSx	-29.2	-21.2	-98.1	0
ASO2570	AluJb	-21.4	-63.4	-19.8	-68.6
ASO2576	FLAM_C	0	0	-28.1	0
	tRNA-Ala-				
ASO2580	GCY_	-25.5	0	-40.1	0
ASO2593	FRAM	-22.9	-52.3	0	-38.1
ASO2601	AluSx	0	-21.2	-47.3	-23
ASO2603	AluJb	-49.5	-90.4	0	-105.2
ASO2605	FRAM	-50.5	-88.5	-13.2	-69
ASO2608	AluJb	-34.2	-40.3	-20.3	-50.1
ASO2610	AluSx	-29.2	-21.2	-98.1	0
ASO2616	AluSx	0	-77.7	0	-29.3
ASO2617	AluJb	0	-73.5	0	-30.2
ASO2620	AluJb	-35.9	-56.8	0	-64.5
ASO2633	AluSx	0	-78.8	-18.5	-64
ASO2634	AluSx	-51.4	-87.7	-22.2	-65.3
ASO2640	AluSx	-33.4	-34.2	-14.9	-36.7
ASO2643	FLAM_C	-58.7	-66.3	0	-64.9
ASO2659	FLAM_C	-16.1	-69.5	0	-50
ASO2665	AluJb	0	0	-41.9	0
ASO2672	AluSx	0	0	-85.3	-19.9
ASO2673	AluJb	0	-22.1	-27.7	-26.3
ASO2674	FLAM_C	0	0	-45.5	0
ASO2684	FLAM_C	0	-61.4	-15.7	-61.1
ASO2689	AluJb	-38	-76.7	-25.8	-72.3
ASO2704	AluSx	-31.4	-96.2	0	-84.5
ASO2713	AluJb	-48.6	-100.7	-10.7	-93.4
ASO2719	FLAM_C	-49.7	-97.9	0	-50.6
ASO2725	AluSx	-58.1	-114.4	-22.2	-90.4
ASO2741	AluJb	-12.1	-68.5	0	-75.8
ASO2753	FLAM_C	-60.3	-113.9	0	-72.3
ASO2759	SVA_A	-22.9	0	0	-73.7
ASO2768	AluSx	0	-13	-70.9	-20.5
ASO2769	AluSx	0	0	-81.5	0
ASO2774	AluJb	-13.9	-73.1	-18.6	-66.6
ASO2776	FRAM	0	-99.6	0	-86.6
ASO2785	FLAM_C	-17.7	0	-54.6	0
ASO2806	AluSx	-48.6	-106.5	-13.6	-57.6
ASO2815	FRAM	-20.3	-20	-48.5	-21.9
ASO2834	AluY	0	0	-39.2	-18.7
ASO2846	AluJb	-24.6	0	-74	0
	tRNA-Ala-				
ASO2867	GCY_	0	0	0	-22.9
ASO2883	AluSx	0	-25.4	-85.4	-40.4
ASO2894	AluSx	0	-21.7	0	-33.4
ASO2897	AluJb	-47.2	-100.5	-25.9	-86.3
ASO2907	AluSx	0	0	-20.1	0
ASO2911	AluJb	-48.6	-64.3	-11.3	-53.9
ASO2913	FRAM	-28.4	-30.6	-50.6	0
ASO2930	AluJb	-38	-74.7	-31.9	-39

ASO2931	AluJb	-20.8	-109.6	-23.7	-104.3
ASO2936	tRNA-Ala-GCY_	0	0	0	0
ASO2941	tRNA-Ala-GCY_	0	0	0	0
ASO2951	tRNA-Ala-GCA	0	0	0	0
ASO2966	AluJb	-46.8	-110.7	-13.2	-95.9
ASO2981	AluSx	-50.9	-103.1	0	-96.8
ASO3003	AluJb	-24	-20.8	-110.2	0
ASO3015	AluSx	-25.6	-21.2	-37.7	0
ASO3025	AluJb	0	0	-56.1	-18.9
ASO3037	AluJb	-18.4	-42.5	-26.6	-52
ASO3061	AluJb	0	-21.2	-111	0
ASO3064	FLAM_C	0	0	-60.3	0
ASO3070	tRNA-Ala-GCY_	0	0	-9.9	0
ASO3086	AluSx	0	0	-30.1	0
ASO3093	FLAM_C	-49.7	-70.8	0	-59.4
ASO3103	FLAM_C	-30.9	-52	-20.1	-46.7
ASO3111	FRAM	-43	-89.5	0	-69.7
ASO3112	AluJb	0	0	-57.2	-24.3
ASO3116	AluJb	0	0	-46.9	0
ASO3137	FLAM_C	-52.7	-112.4	0	-82.2
ASO3154	AluJb	-48.3	-99.4	0	-86.6
ASO3167	AluJb	-32.2	-51.7	0	-59.9
ASO3179	tRNA-Ala-GCY_	0	-18.9	-18.7	0
ASO3181	tRNA-Ala-GCY_	0	0	-12.7	0
ASO3183	FLAM_C	0	-22.4	-47.8	-15.8
ASO3200	AluSx	-25	-76.1	-18.6	-71.2
ASO3209	FRAM	-13.5	0	-77.3	0
ASO3222	FLAM_C	0	0	-52.2	-26.8
ASO3245	AluJb	-45	-71.8	0	-67.1
ASO3260	FLAM_C	0	0	-33.6	0
ASO3267	AluJb	0	-46.1	0	-24.6
ASO3268	AluJb	-23	-34.2	0	-43
ASO3280	tRNA-Thr-ACA	-16.7	-16.8	-18.5	0
ASO3281	AluJb	0	-21.8	-85.3	0
ASO3286	FLAM_C	-20.1	-29.3	-26.3	-50.1
ASO3290	AluJb	-17.2	-107.2	-11.3	-95.9
ASO3300	AluSx	-51.3	-114.4	0	-99.9
ASO3303	FLAM_C	-53.7	-20.6	0	-43.6
ASO3304	FLAM_C	-11.2	-76.3	-11.2	-61.2
ASO3307	AluJb	-24	-27.9	-102.1	-24.1
ASO3310	FLAM_C	0	-19.9	-69.8	-21
ASO3329	FRAM	-23	-50.1	-30.4	-42.3
ASO3334	FRAM	-49	-83	0	-8.7
ASO3347	AluJb	0	-18.7	-24.8	0
ASO3351	AluSx	0	-59.7	-30.3	-23.2
ASO3355	AluSx	0	0	-61.6	-17.8
ASO3357	AluSx	0	-39	-25.5	-40.3
ASO3360	BC200	-63.7	-35.2	-20.1	-35.8
ASO3363	tRNA-Ala-	0	0	0	-29.6

ASO3365	GCY_ tRNA-Ala- GCA	0	0	0	0
ASO3369	GCY_ tRNA-Ala- AluSx	-25.6	0	0	0
ASO3378	GCY_ tRNA-Ala- GCA	0	-22.4	-59.1	0
ASO3383	GCY_ AluJb	0	-31.3	0	-25.7
ASO3389	AluJb	0	-21.8	-53.6	-19.2
ASO3390	FLAM_C	-32.9	-62.4	0	-25.1
ASO3397	FLAM_C	0	0	-41.5	0
ASO3406	AluY	-42.6	-73.1	0	-85.2
ASO3413	AluJb	-40.7	-104.3	-21	-94.6
ASO3428	AluSx	0	0	-321.7	-25.6
ASO3436	AluJb	-85	-97.7	-34.7	-126.7
ASO3442	AluJb	-118.6	-189.7	0	-106.4
ASO3455	FLAM_C	-100.3	-46.3	0	-49.5
ASO3457	AluSx	-118.5	-382.1	-25.2	-253
ASO3459	AluJb	-75.1	-94.2	-20.3	-74.7
ASO3462	AluY	0	0	-205.2	-29.2
ASO3466	AluJb	-138.4	-155	-24.1	-183.9
ASO3467	FLAM_C	-99.8	-74.3	0	-71.5
ASO3470	AluSx	0	0	-89	-19.6
ASO3476	AluY	0	-31.1	-117.9	-25.7
ASO3478	AluSx	0	0	-135.9	0
ASO3481	AluJb	0	-27.7	-150.2	-18.7
ASO3488	AluSx	-66.4	-261.9	-19.1	-203.1
ASO3491	AluJb	-101.9	-156.1	-22.2	-142.2
ASO3493	AluSx	-122	-263.5	0	-252.5
ASO3498	AluJb	0	-26.9	-198.5	-34.7
ASO3502	AluJb	-29.9	-29.8	-96.7	-31.2
ASO3505	AluSx	-61.3	-116	-21.1	-142.5
ASO3506	AluY	-49.9	-150.9	0	-153.9
ASO3517	AluSx	0	-17.2	-216.8	-36.6
ASO3520	AluSx	0	0	-118.6	0
ASO3521	AluSx	0	0	-118.6	0
ASO3528	AluY	-62.7	-326.8	-18.1	-403.8
ASO3534	AluSx	0	-30.6	-166.4	-15.5
ASO3535	AluJb	-118.2	-88.2	-20.6	-107.8
ASO3539	AluSx	0	0	-93	0
ASO3545	FRAM	-59.2	-66.7	-39.3	-85.4
ASO3546	AluSx	-98.4	-352.8	-26.7	-144
ASO3548	AluSx	-101.8	-214.3	0	-163.2
ASO3556	AluSx	-125.2	-223.9	-34.2	-128.7
ASO3557	AluY	0	0	-100.8	0
ASO3563	AluSx	-80.9	-124.3	-18.8	-145.5
ASO3566	FRAM	-41.7	-44.4	0	-82.3
ASO3573	FRAM	-42.8	-56.2	-33	-55
ASO3583	AluSx	0	0	-139	-29.2
ASO3584	AluSx	0	0	-149.3	-31.3
ASO3586	FLAM_C	-99.6	-83.9	0	-59.4
ASO3593	AluSx	-109.1	-245.4	-18.8	-245.8
ASO3595	AluSx	-65.5	-111.8	0	-125
ASO3601	AluSx	0	0	-82.9	0
ASO3604	FRAM	-87.8	-65	0	-78.3

ASO3606	AluSx	-117.8	-362.6	-14.5	-248.3
ASO3609	AluY	0	-31.9	-192	-19.3
ASO3629	AluSx	0	0	-211.8	0
ASO3630	AluSx	-95.6	-420.4	-17.1	-210.7
ASO3633	FLAM_C	-54.9	-96.3	0	-112.2
ASO3635	AluSx	-74.1	-220.9	-43.4	-163.2
ASO3645	AluSx	0	0	-214	-39.8
ASO3661	AluSx	0	-89.9	-10.6	-72.5
ASO3667	AluSx	0	-19.6	-153.7	-26.3
ASO3668	AluSx	-45.7	-110.5	-42.2	-100.3
ASO3673	AluY	0	0	-71.1	0
ASO3674	FLAM_C	0	-22.8	-60.8	0
ASO3680	FLAM_C	-118.4	-178.6	-23.2	-77.3
ASO3682	AluJb	-62.4	-85.8	0	-119.4
ASO3690	AluSx	-74.1	-220.9	-43.4	-163.2
ASO3695	AluSx	0	0	-104.4	0
ASO3698	AluSx	-12.5	0	-124.8	0
ASO3710	FLAM_C	-99.8	-77.7	0	-59.6
ASO3717	AluSx	0	0	-100.3	0
ASO3720	AluJb	-155.7	-182.1	-11.4	-89.8
ASO3721	AluSx	0	0	-118.6	0
ASO3722	AluSx	-65.5	-111.8	0	-125
ASO3735	AluSx	0	0	-118.6	0
ASO3739	AluSx	0	-19.8	-207	-25.6
ASO3742	AluSx	0	-29.8	-231.9	-16.3
ASO3745	AluSx	-86.5	-350.5	-25.8	-260.7
ASO3746	AluSx	0	-34.7	-211.3	-20.8
ASO3747	AluJb	-50.4	-98.4	-11.4	-68.1
ASO3755	FLAM_C	0	0	-98.3	-27.2
ASO3761	AluSx	-61.3	-109.1	0	-119.2
ASO3780	AluSx	-66.5	-127.9	-20.1	-134
ASO3803	AluSx	-124.7	-308.8	-20.4	-202
ASO3804	FLAM_C	-120.1	-124.7	0	-75.9
ASO3809	AluSx	0	0	-118.6	0
ASO3813	AluSx	-13.9	0	-157.2	-13.1
ASO3817	FRAM	-60.8	-97.6	-22.6	-107.7
ASO3820	AluJb	0	0	-100.4	-18.4
ASO3821	AluJb	-82.1	-178.7	-16.3	-101.7
ASO3822	AluSx	0	0	-163.3	-18.9
ASO3837	AluJb	-122.4	-163.1	-8	-142.8
ASO3838	AluSx	0	0	-118.6	0
ASO3847	AluY	-47.3	-173.4	-26.4	-167.5
ASO3851	AluSx	-112.6	-304.3	-34.7	-172.8
ASO3861	AluJb	-134.1	-177.1	-16.3	-78.2
ASO3864	AluSx	-107.6	-235.8	-30.3	-249.5
ASO3872	FLAM_C	0	0	-124.5	0
ASO3873	AluSx	-65.2	-207.1	-20.8	-342
ASO3874	AluSx	0	0	-159.1	-16.3
ASO3875	AluSx	0	0	-118.6	0
ASO3876	AluJb	0	0	-71.1	0
ASO3878	AluY	0	0	-112.6	0
ASO3881	AluJb	-99.6	-92.1	-19.2	-120.7
ASO3882	FLAM_C	0	-30.9	-68.7	0
ASO3891	AluSx	-13.5	-17.2	-127.7	-17.6
ASO3893	AluY	-69.7	-202.2	-24.6	-404.1

ASO3900	AluJb	-91.4	-119.5	0	-89.1
ASO3902	AluSx	0	-15.6	-189.1	-27
ASO3906	AluSx	0	0	-159.1	-16.3
ASO3908	AluJb	-69.8	-157.8	-28.7	-88.9
ASO3911	AluSx	0	-28.8	-302.9	-21.1
ASO3913	AluSx	-98.4	-352.8	-26.7	-144
ASO3946	AluJb	-75.1	-94.2	-20.3	-74.7
ASO3947	AluJb	-150.2	-214.4	-22.2	-106.5
ASO3948	AluY	0	0	-115.4	0
ASO3950	FRAM	-67.6	-103.4	-13.6	-64.1
ASO3952	AluSx	0	-26.9	-105.7	-25
ASO3960	AluSx	-86.5	-350.5	-25.8	-260.7
ASO3961	AluSx	-111.4	-360.3	-39.9	-262.4
ASO3973	AluJb	-32	0	-298.5	0
ASO3974	AluSx	-99	-366.7	-36.2	-262.2

*From Antisense lncRNA Pipeline

**Motif search using REPEATMASKER for Alu sequences within the specific lncRNA

***Free energy (DG) calculation considered duplexes that contain ≥ 10 continuous basepairs; otherwise, free energy was designated to be 0.

- 5 Grey highlights denote studied lncRNAs: ASO1998, $\frac{1}{2}$ -sbsRNA4; ASO3488, $\frac{1}{2}$ -sbsRNA3; ASO3667, $\frac{1}{2}$ -sbsRNA2; ASO3720, $\frac{1}{2}$ -sbsRNA1

Among them, the Alu element of lncRNA_AF087999 (NCBI) has the potential to base-pair with the Alu element within SERPINE1 and FLJ21870 3'UTRs (Fig. 3a; Fig. 4a) with ΔG values of, respectively, -151.7 kcal/mol and -182.1 kcal/mol (Table 9; where -151.7 kcal/mol defined the most stable duplex predicted to form between SERPINE1 mRNA and any of the 378 lncRNAs). lncRNA_AF087999, which for reasons that follow is designated $\frac{1}{2}$ -sbsRNA1, derives from chromosome 11. RT-semiquantitative (sq)PCR demonstrated that $\frac{1}{2}$ -sbsRNA1 is detected in cytoplasmic but not nuclear HeLa-cell fractions and is polyadenylated. Downregulating the cellular abundance of the two major isoforms of STAU1 to $<10\%$ of normal (see, e.g., below) did not affect either the cellular distribution or the abundance of $\frac{1}{2}$ -sbsRNA1. $\frac{1}{2}$ -sbsRNA1 is present in every human tissue that was examined. $\frac{1}{2}$ -sbsRNA1 is not a substrate for Dicer or AGO2 and thus is distinct from the lncRNAs that generate endogenous siRNAs.

- 20 203. Two forms of $\frac{1}{2}$ -sbsRNA1 have been reported (NCBI). They differ at their 5' end but share a common Alu element and a common 3' end that contains a putative polyadenylation signal (AUUAAA) situated 13 nucleotides upstream of a poly(A) tract. RNase protection assays confirmed the presence of one short (S) and one long (L) form of $\frac{1}{2}$ -sbsRNA1 that have a different 5' end and a relative abundance in HeLa cells of 3:1 (Fig. 25 5a). Primer extension (Fig. 5b) and RT-sqPCR (Fig. 5c) mapped the 5' end of $\frac{1}{2}$ -sbsRNA1(S) to a C residue. Therefore, $\frac{1}{2}$ -sbsRNA1(S) consists of 688 nucleotides

excluding the poly(A) tract (Fig. 5d). While some transcripts that are annotated as ncRNAs may be translated, data indicate that $\frac{1}{2}$ -sbsRNA1(S) is not translated.

204. Remarkably, not only STAU1 siRNA but also $\frac{1}{2}$ -sbsRNA1 siRNA increased the levels of SERPINE1 and FLJ21870 mRNAs to 2-to-4.5-fold above normal (Fig. 3b and
5 Table 10).

Table 10: Comparison of RT-sqPCR shown in Fig. 1 and RT-qPCR *

	siRNA	RT-sqPCR **			RT-qPCR		
		Control	STAU1	$\frac{1}{2}$ -sbsRNA1	Control	STAU1	$\frac{1}{2}$ -sbsRNA1
Fig. 1b	$\frac{1}{2}$ -sbsRNA1	100	103±5	61±4	100	107±4	64±3
	SERPINE1 mRNA	100	451±5	308±5	100	443±7	336±10
	FLJ21870 mRNA	100	325±15	200±10	100	303±22	201±2
Fig. 1c	FLUC-SERPINE1 3'UTR mRNA	13±5	82±7	44±3	11±7	82±4	48±4
	FLUC-FLJ21870 3'UTR mRNA	11±3	93±7	65±6	16±5	92±6	68±3

* sqPCR and qPCR used the same RT reactions

** See Fig. 3c legend for details

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Furthermore, experiments that employed cycloheximide indicated that the $\frac{1}{2}$ -sbsRNA1-mediated reduction in SERPINE1 and FLJ21870 mRNA abundance depends on translation, as does SMD. The reduction in SERPINE1 and FLJ21870 mRNA abundance is attributable to their respective 3'UTR sequences since $\frac{1}{2}$ -sbsRNA1 siRNA also increased the levels of
15 FLUC-SERPINE1 3'UTR and FLUC-FLJ21870 3'UTR reporter mRNAs relative to FLUC-No SBS mRNA (Fig. 3c and Table 10). The $\frac{1}{2}$ -sbsRNA1 siRNA-mediated increase in the abundance of SERPINE1 or FLJ21870 mRNA was reversed by co-expressing $\frac{1}{2}$ -sbsRNA1(S)R, which is resistant to siRNA, arguing against siRNA-mediated off-target effects. Significantly, $\frac{1}{2}$ -sbsRNA1 siRNA did not affect the expression of other FLUC
20 reporter mRNAs that contain the 3'UTR of SMD targets not predicted to base-pair with $\frac{1}{2}$ -sbsRNA1.

205. If $\frac{1}{2}$ -sbsRNA1 were to create an SBS by base-pairing with the 3'UTR of SERPINE1 or FLJ21870 mRNA, then it should be possible to co-immunoprecipitate complexes of the lncRNA and each mRNA. To test this possibility, lysates of HeLa cells
25 that transiently expressed (i) $\frac{1}{2}$ -sbsRNA1(S)-MS2bs, which contains 12 copies of the MS2 coat protein binding site (MS2bs) upstream of the lncRNA polyadenylation signal or, as a negative control, $\frac{1}{2}$ -sbsRNA1(S) or FLUC-MS2bs mRNA (Fig. 3d) and (ii) FLAG-MS2-hMGFP, which consists of FLAG-tagged MS2 coat protein fused to hMGFP, were immunoprecipitated using anti-FLAG. As expected, prior to IP $\frac{1}{2}$ -sbsRNA1(S) as well as

$\frac{1}{2}$ -sbsRNA1(S)-MS2bs decreased the abundance of SERPINE1 and FLJ21870 mRNAs but not SMD targets that encode interleukin 7 receptor (IL7R), CUG domain-containing protein 1 (CDCP1) or methylthioadenosine phosphorylase (MTAP) (Fig. 3e; see below). In support of the finding that $\frac{1}{2}$ -sbsRNA1 creates an SBS with partially complementary mRNA sequences, using lysates of cells expressing $\frac{1}{2}$ -sbsRNA1(S)-MS2bs, the anti-FLAG IP of FLAG-MS2-hMGFP bound to $\frac{1}{2}$ -sbsRNA1(S)-MS2bs co-immunoprecipitated endogenous STAU1, SERPINE1 mRNA and FLJ21870 mRNA as well as the UPF1 SMD factor (Fig. 3e). In contrast, irrelevant proteins, such as Calnexin, the dsRNA binding protein ILF3, the single-stranded RNA binding protein FMR1, and mRNAs that are not predicted to base-pair with $\frac{1}{2}$ -sbsRNA1, such as those encoding SMG7, IL7R, CDCP1 or MTAP, were not coimmunoprecipitated (Fig. 3e). STAU1 siRNA reduced the co-IP of the SERPINE1 mRNA as well as FLJ21870 mRNA with $\frac{1}{2}$ -sbsRNA1(S)-MS2bs to, respectively, ~19% or ~15% of normal (Fig. 3f), indicating that STAU1 stabilizes the duplex formed between SERPINE1 or FLJ21870 mRNA and $\frac{1}{2}$ -sbsRNA1.

206. As additional evidence that $\frac{1}{2}$ -sbsRNA1 creates an SBS by base-pairing with the SERPINE1 or FLJ21870 3'UTR, only STAU1-HA3 but not ILF3 or FMR1 coimmunoprecipitated with $\frac{1}{2}$ -sbsRNA1.

207. To determine if $\frac{1}{2}$ -sbsRNA1 is required for the co-IP of STAU1 with SERPINE1 or FLJ21870 mRNA, HeLa cells that transiently expressed STAU1-HA3 and Control siRNA or $\frac{1}{2}$ -sbsRNA1 siRNA in the presence or absence of $\frac{1}{2}$ -sbsRNA1(S)R were immunoprecipitated using anti-HA. Compared to Control siRNA, $\frac{1}{2}$ -sbsRNA1 siRNA, which reduced the level of $\frac{1}{2}$ -sbsRNA1 to ~50% of normal, reduced by ~2-fold the co-IP of STAU1-HA3 with SERPINE1 or FLJ21870 mRNA (Fig. 6a). In contrast, restoring the level of $\frac{1}{2}$ -sbsRNA1 to ~100% of normal by expressing $\frac{1}{2}$ -sbsRNA1 siRNA together with $\frac{1}{2}$ -sbsRNA1(S)R restored the co-IP of STAU1-HA3 with SERPINE1 or FLJ21870 mRNA to near normal (Fig. 6a). As expected, the level of IL7R mRNA, which binds STAU1 but does not contain sequences complementary to $\frac{1}{2}$ -sbsRNA1, was unaffected by any condition either before or after IP (Fig. 6a).

208. Thus, the SMD of SERPINE1 or FLJ21870 mRNA involves basepairing between their 3'UTR Alu element and the Alu element within $\frac{1}{2}$ -sbsRNA1. Basepairing creates an SBS that is stabilized by STAU1. Furthermore, the level of STAU1 and, thus, the efficiency of SMD does not alter the level of $\frac{1}{2}$ -sbsRNA1. The finding that downregulating SERPINE1 or FLJ21870 mRNA to 50% and 25% of normal, respectively, failed to

detectably decrease the co-IP of STAU1-HA3 with ½-sbsRNA1 (Fig. 7) indicates that ½-sbsRNA1 can bind to more than SERPINE1 and FLJ21870 mRNAs to recruit STAU1 if not trigger SMD.

209. The presence of UPF1 in the anti-FLAG IP of FLAG-MS2-hMGFP (Fig. 3e) is consistent with the idea that STAU1 that is bound to a ½-sbsRNA1-created SBS associates with UPF1, analogously to how STAU1 that is bound to the ARF1 SBS associates with UPF1. Furthermore, downregulating UPF1, like downregulating STAU1, increases the abundance of SERPINE1 mRNA, FLJ21870 mRNA and FLUCSERPINE1 3'UTR mRNA by increasing mRNA half-life. To test for UPF1 function in conjunction with ½-sbsRNA1, the effects of various siRNAs on the production of FLUC-SERPINE1 3'UTR mRNA in which the 3'UTR was intact, precisely lacked the region that was partially complementary to ½-sbsRNA1, or contained solely this region (Fig. 6b) were analyzed. Relative to Control siRNA, STAU1 siRNA, UPF1 siRNA or ½-sbsRNA1 siRNA did not affect the level of FLUC-SERPINE1 3'UTR mRNA that lacked the ½-sbsRNA1 binding site (Fig. 6c), but each siRNA increased the levels of FLUC-SERPINE1 3'UTR mRNA and FLUC mRNA that contained only the ½-sbsRNA1-BS (Fig. 6c). Thus, as indicated by its name, ½-sbsRNA1 base-pairs with the 3'UTR of SERPINE1 mRNA and, by analogy, FLJ21870 mRNA so as to recruit STAU1 and its binding partner UPF1 in a way that triggers a reduction in mRNA abundance. Consistent with previous studies of SMD, the STAU1- and ½-sbsRNA1-mediated reduction in mRNA abundance is due to a decrease in mRNA half-life. With regard to function, scrape injury repair assays revealed that ½-sbsRNA1 contributes toward reducing cell migration by targeting SERPINE1 and RAB11FIP1 mRNAs for SMD (Fig. 8).

210. Characterizing seven other lncRNAs that contain a single Alu element and consist of <1000 nucleotides (Table 9) confirmed that they, too, are largely cytoplasmic and polyadenylated and have the potential to base-pair with the single Alu element within at least one mRNA 3'UTR (Fig. 9a; Fig. 4b,c,d; Table 9). Individually downregulating three of these lncRNAs – lncRNA_BC058830 (½-sbsRNA2), lncRNA_AF075069 (½-sbsRNA3) or lncRNA_BC009800 (½-sbsRNA4) – upregulated those tested mRNAs that (i) contain a partially complementary Alu element and (ii) are upregulated upon STAU1 or UPF1 downregulation; each lncRNA failed to upregulate mRNAs that lack a partially complementary Alu element (Fig. 9b). While ½-sbsRNA2 targeted the 3'UTR Alu element of CDCP1 mRNA (Fig. 9b and Table 9, where $\Delta G = -153.7$ kcal/mol), ½-sbsRNA3 and ½-

sbsRNA4 targeted the 3'UTR Alu element of MTAP mRNA (Fig. 9b and Table 9, where $\Delta G = -203.1$ and -264.2 kcal/mol, respectively). Furthermore, none of the three lncRNAs downregulated SERPINE1 mRNA (Fig. 9b and Table 9, where $\Delta G = 0$, -66.4 and -108.2 kcal/mol, respectively) but two downregulated FLJ21870 mRNA ~2-fold (Fig. 9b and Table 9, where $\Delta G = -261.9$ and -444.2 kcal/mol).

211. These findings illustrate the potentially complex network of regulatory events that are controlled by lncRNA-mRNA duplexes that bind STAU1 and is reminiscent of the web of regulatory mechanisms that are mediated by miRNAs. Notably, both CDCP1 mRNA and MTAP mRNA were upregulated at least 2-fold upon STAU1 downregulation in experiments disclosed herein (Fig. 9b), and indeed CDCP1 mRNA is among those mRNAs that were upregulated minimally 1.8-fold upon STAU1 downregulation (Table 8). However, since MTAP mRNA was upregulated only ~1.5-fold, it is not included in Table 8.

212. It is important to note that ΔG values are not in themselves absolute predictors of SBS function. For example, while $\frac{1}{2}$ -sbsRNA2 is predicted to base-pair with the 3'UTR Alu element of BAG5 mRNA with a ΔG of -416 kcal/mol, BAG5 mRNA is not targeted for SMD in HeLa cells. The 3'UTR Alu element of BAG5 mRNA may be physically inaccessible to base-pairing with $\frac{1}{2}$ -sbsRNA2. Nevertheless, base-pairing per se may not be sufficient for SBS function since converting the 100-nt apex of the intramolecular ARF1 SBS to a 4-nt loop that is not predicted to disrupt the adjacent 19-bp stem of the ARF1 SBS reduces STAU1 binding *in vivo* by 50%.

213. Herein is disclosed a previously unforeseen role for some of the lncRNAs that contain Alu elements: the creation of SBSs by intermolecular base-pairing with an Alu element within the 3'UTR of one or more mRNAs. Thus, SBSs can form either through intramolecular base-pairing, as exemplified by the ARF1 SBS, or intermolecular basepairing between a $\frac{1}{2}$ -SBS within an mRNA 3'UTR and a complementary $\frac{1}{2}$ -sbsRNA in the form of a largely cytoplasmic lncRNA (Fig. 9c).

214. There are estimated to be tens of thousands of human lncRNAs that have little or no ability to direct protein synthesis and that are distinct from rRNAs, tRNAs, snRNAs, snoRNAs, small interfering RNAs or microRNAs. Thus, the paradigm that partially complementary ncRNA-mRNA duplexes can form SBSs can extend to the creation of binding sites for other dsRNA binding proteins. Since only 23% of lncRNAs were found to contain one or more Alu elements, lncRNA-mRNA duplexes that do not

involve Alu elements expand the number of ncRNAs that regulate gene expression via SMD or a different dsRNA binding protein-dependent pathway.

Example 7: Computational analyses.

215. The Perl program “Alu_Mask” was designed to define Alu elements within
5 known and putative SMD targets and ncRNAs based on results obtained using REPEATMASKER.

216. The Perl program “RNA_RNA_anneal” was developed to predict Alu-element base-pairing between lncRNA_AF087999 (1/2-sbsRNA1) and the SERPINE1 or FLJ21870 mRNA 3'UTR, lncRNA_BC058830 (1/2-sbsRNA2) and the CDCP1 mRNA
10 3'UTR, as well as lncRNA_AF075069 (1/2-sbsRNA3) or lncRNA_BC009800 (1/2-sbsRNA4) and the MTAP or FLJ21870 mRNA 3'UTR. Potential duplexes were first fixed using what was predicted to be the most stably and perfectly base-paired region and then expanded in both directions, allowing for bulges or loops of up to 10 nucleotides until base-pairing was no longer predicted. Briefly, “RNA_RNA_anneal” uses a recursive algorithm that predicts
15 the most stable base-pairs and their ΔG value based on thermodynamic data that were extracted from RNA Structure 4.6. Duplexes between other ncRNAs and mRNA 3'UTRs were likewise predicted using this approach. All data from “RNA_RNA_anneal” were validated using RNA structure 4.6, which provides folding free energy changes.

217. Notably, to follow up the finding that ~13% of the ~1.6% of HeLa-cell
20 proteinencoding transcripts that are upregulated at least 1.8-fold upon STAU11,2 contain a single Alu element, a random resampling of 1.6% of total-cell mRNAs (NCBI) 10,000 times revealed that the presence of one or more Alu elements in the 3'UTRs of potential SMD targets (Table 8) was enriched ~3.58-fold ($p < 0.001$).

Example 8: Plasmid Constructions.

218. To construct pcDNA3.1(+)/Zeo_Chr11_66193000-66191383, HeLa-cell
25 genomic DNA was purified using DNeasy Blood & Tissue Kit (Qiagen) and PCR-amplified using the primer pair: 5'- GATGCTCGAGTGGCATTGGCTTTTACCACCTATG-3' (sense) (SEQ ID NO: 51) and 5'- GTCAGGATCCTGCCTCAAGTCCAAAGCACAACCTG-3' (antisense) (SEQ ID NO: 52), where underlined nucleotides specify a XhoI or BamHI
30 site. The resulting PCR product was cleaved with XhoI and BamHI and inserted into XhoI- and BamHI-cleaved pcDNA3.1(+)/Zeo (Invitrogen).

219. To generate p1/2-sbsRNA1(S) or p1/2-sbsRNA1(S)-MS2bs, pcDNA3.1(+)/Zeo_Chr11_66193000-66191383 was amplified using the primer pair 5'-

GAGTCAAAGCTTAAAGGAGAGACAGTCTCACTCTG-3' (sense) (SEQ ID NO: 53) and, respectively, 5'-GTCAGCGGCCGCCAGTTGTAAGCATATTTGGGTAC-3' (antisense) (SEQ ID NO: 54) or 5'-

GTCAGGATCCAGTTGTAAGCATATTTGGGTAC-3' (antisense) (SEQ ID NO: 55),

5 where underlined nucleotides denote a HindIII, NotI or BamHI site. The resulting PCR products were cleaved with HindIII and either NotI or BamHI, respectively, and inserted into HindIII- and NotI-cleaved or HindIII- or BamHI-cleaved pcDNA3-MS2bs.

220. Overlap-extension PCR was used to construct p $\frac{1}{2}$ -sbsRNA1(S)R. Two rounds of site-directed mutagenesis were performed using p $\frac{1}{2}$ -sbsRNA1(S) and the primer pairs 5'-

10 GATATTCATTACTAACCCCTGAACCCATACAGTTCAGCTTACCACTACAGTACTTCT-3' (sense) (SEQ ID NO: 56) and 5'-

GAAGTACTGTAGTGGTAAGCTGAACTGTATGGGTTCAGGGGTTAGTAATGAATATC-3' (antisense) in the first round, and 5'-

15 CCTGAACCCATACAGTTCAGCTCAGAACTACAGTACTTCTGTAGT-3' (sense) (SEQ ID NO: 57) and 5'-

ACTACAGAAGTACTGTAGTTCTGAGCTGAACTGTATGGGTTCAGG-3' (antisense) (SEQ ID NO: 58) in the second round, where mutagenic nucleotides are underlined.

221. To generate pFLUC-MS2bs, pcFLUC was PCR-amplified using the primers pair 5'-GAGTCAAAGCTTATGGAAGACGCCAAAAACATAAAGAAAGGC-3' (sense) (SEQ ID NO: 59) and 5'-GTCAGGATCCTTACAATTTGGACTTTCCGCCCTTCTTGGC-3' (antisense) (SEQ ID NO: 60), where underlined nucleotides specify a HindIII or BamHI site. The resulting PCR product was digested with HindIII and BamHI and inserted into HindIII- and BamHI-cleaved pcDNA3-MS2bs.

222. To construct pFLAG-MS2-hMGFP, pMS2-HA was amplified using the primer pair 5'-

25 GATGGCTAGCCGCCATGGACTACAAAGACGATGACGACAAGGGATCCGCTTCTAACTTTACTCAGTTTCG-3' (sense) (SEQ ID NO: 61) and 5'-

GTCAGATATCGTAGATGCCGGAGTTTGCTGCG-3' (antisense) (SEQ ID NO: 62),

30 where underlined nucleotides specify a NheI or EcoRV site. The resulting PCR product was digested using NheI and EcoRV and inserted into NheI- and EcoRV-cleaved pHMGFP (Promega).

223. To create p^{1/2}-sbsRNA1(S)-hMGFP, phMGFP was amplified using the primer pair 5'-GATGCCTAGGGGCGTGATCAAGCCCGACATG-3' (sense) (SEQ ID NO: 63) and 5'-GTCACCTAGGGCCGGCCTGGCGGGGTAGTCC-3' (antisense) (SEQ ID NO: 64), where underlined nucleotides identify the AvrII site. The resulting PCR product was
5 digested with AvrII and inserted into the AvrII site of p^{1/2}-sbsRNA1(S).

224. To construct pFLUC-FLJ21870 3'UTR, two fragments of the FLJ21870 3'UTR were amplified using HeLa-cell genomic DNA and the primer pair 5'-GATGTCTAGAGTGATCAACTTCGCCAACAAACACCAG-3' (sense) (SEQ ID NO: 65) and 5'-CAGAAGGCTAGCCCGAAGAGAAC-3' (antisense) (SEQ ID NO: 66), or 5'-
10 CTCTTCGGGCTAGCCTTCTGG-3' (sense) (SEQ ID NO: 67) and 5'-GTCAGGGCCCGAGACAGAGTCTCCGTTGCCC-3' (antisense) (SEQ ID NO: 68), where underlined nucleotides denote a XbaI, NheI or ApaI site. The resulting PCR fragments were digested using NheI and either XbaI or ApaI, respectively, and inserted simultaneously into pFLUC-SERPINE1 3'UTR5 that had been digested with XbaI and
15 ApaI.

225. To create pFLUC-SERPINE1 3'UTR Δ(^{1/2}-sbsRNA1-BS), two regions of the SERPINE1 3'UTR were amplified using pFLUC-SERPINE1 3'UTR and primer pairs 5'-GAGTCAAAGCTTGGCATTCCGGTACTGTTGG-3' (sense) (SEQ ID NO: 69) and 5'-CATCCATCTTTGTGCCCTACCC-3' (antisense) (SEQ ID NO: 70) or 5'-
20 TCTTTAAAAATATATATATTTTAAATATAC-3' (sense) (SEQ ID NO: 71) and 5'-TAGAAGGCACAGTCGAGG-3' (antisense) (SEQ ID NO: 72), where underlined nucleotides denote a HindIII site. The resulting PCR fragments were phosphorylated using T4 polynucleotide kinase, digested with HindIII or ApaI, respectively, and inserted simultaneously into pFLUC-SERPINE1 3'UTR that had been digested with HindIII and
25 ApaI.

226. To generate pFLUC-SERPINE1 ^{1/2}-sbsRNA1-BS, the ^{1/2}-sbsRNA1-BS was amplified using pFLUC-SERPINE1 3'UTR and the primer pair 5'-GATGTTTAAATAATGCACTTTGGGAGGCCAAGG-3' (sense) (SEQ ID NO: 73) and 5'-GATGTTTAAAGACGGGGGTCTTGGTATGTTGC-3' (antisense) (SEQ ID NO: 74),
30 where underlined nucleotides denote a DraI site. The resulting PCR product was then digested with DraI. Meanwhile, pFLUC No SBS was digested with HindIII and ApaI, and the released the FLUC No SBS region was subsequently digested with DraI. All three fragments from the pFLUC No SBS digestions were then ligated to the PCR product.

227. To generate pTRE-FLUC-SERPINE1 3'UTR or pTRE-FLUC-FLJ21870 3'UTR, FLUC-SERPINE1 3'UTR or FLUC-FLJ21870 3'UTR was amplified using, respectively, pFLUC-SERPINE1 3'UTR and the primer pair 5'-GATACCGCGGATGGAAGACGCCAAAAACATAAAG-3' (sense) (SEQ ID NO: 75) and 5'-GTCAGAATTCGCTTCTATTAGATTACATTCATTTTCAC-3' (antisense) (SEQ ID NO: 76), or pFLUCFLJ21870 3'UTR and the primer pair 5'-GATACCGCGGATGGAAGACGCCAAAAACATAAAG-3' (sense) (SEQ ID NO: 77) and 5'-GTCAGAATTCGAGACAGAGTCTCCGTTGCCC-3' (antisense) (SEQ ID NO: 78), where underlined nucleotides denote a SacII or EcoRI site. The resulting PCR product was digested with SacII and EcoRI and inserted into SacII- and EcoRI-cleaved pTRE (Clontech).

Example 9: Cell culture, transient transfection, and formaldehyde crosslinking.

228. Human HeLa or HaCaT cells (2×10^6 /60-mm dish or 7.5×10^7 /150-mm dish) were grown in DMEM (Gibco-BRL) containing 10% fetal bovine serum (Gibco-BRL). Cells were transiently transfected with the specified plasmids using Lipofectamine 2000 (Invitrogen) or the specified siRNA using Oligofectamine (Invitrogen). siRNAs consisted of STAU1 siRNA, $\frac{1}{2}$ -sbsRNA1 siRNA (5'-CCUGUACCCUUCAGCUUACdTdT-3') (SEQ ID NO: 79), $\frac{1}{2}$ -sbsRNA1(A) siRNA (5'-AUGACUUUGGGCAAAGUACdTdT-3') (SEQ ID NO: 80), Dicer1 siRNA (Ambion), AGO2 siRNA (Ambion), SERPINE1 siRNA (Ambion), FLJ21870 siRNA (Ambion), RAB11FIP1 siRNA (Ambion), $\frac{1}{2}$ -sbsRNA2 (5'-GGUGCAAAGACAGCAUUCCdTdT-3') (SEQ ID NO: 81), $\frac{1}{2}$ -sbsRNA3 (5'-UAGUAGUCAAGACCAAUUCUAdTdT-3') (SEQ ID NO: 82), $\frac{1}{2}$ -sbsRNA4 (5'-UGGCAUUCCAGUUGAGUUUdTdT-3') (SEQ ID NO: 83), or the nonspecific Silencer Negative Control #1 (Ambion). Notably, all lncRNA siRNAs used in this study target a sequence outside of the Alu element. For all immunoprecipitations (IPs), cells were crosslinked using 1% formaldehyde for 10 min at room temperature and subsequently quenched with 0.25 M glycine for 5 min at room temperature prior to lysis. In experiments that blocked protein synthesis, cells were incubated with 300 μ g/ml of cycloheximide (Sigma) 3-hr prior to lysis.

229. For mRNA half-life measurements, HeLa Tet-Off cells (Clontech) were transfected with the specified siRNA in the presence of 2 μ g/ml of doxycycline (Clontech). After 48-hr, the medium was replaced to remove doxycycline, and cells were transfected with the indicated reporter and reference plasmids. Four-hr later, an aliquot of cells was

harvested at time 0, 2 µg/ml of doxycycline was added to the remaining cells to silence reporter-gene transcription, and aliquots of cells were harvested as a function of time thereafter.

230. Scrape injury repair assays were essentially as published. Briefly, two days after transfection using siRNA, monolayer cultures of HaCaT cells at 90% confluence in 100-mm dishes were scratched in nine places using a P200 pipette tip (VWR) and uniform pressure to create denuded areas that were 0.9 mm wide. Cells were washed once with growth medium, which removes scratch-generated debris and generates smooth wound edges, and cultured for an additional 16 hr with monitoring.

10 **Example 10: Protein purification, IP and Western blotting.**

231. HeLa cells were lysed, and protein was isolated using hypotonic buffer that consists of 10mM Tris-Cl (pH 7.4), 150mM NaCl, 2mM EDTA, 0.5% Triton X-100, 2mM benzamidine, 1mM PMSF and 1 tablet of protease inhibitor cocktail (Roche). If crosslinked, cells were sonicated six times for 30 sec to facilitate lysis. IP was performed. In experiments that involved formaldehyde crosslinking, crosslinks were reversed by heating at 65°C for 45 min after IP. HeLa cells were cultured as described above but in 150-mm dishes. Transfections and immunopurifications (IPs) were performed as described. Western blotting was performed. Antibodies consisted of anti- STAU1, anti-Calnexin (Calbiochem), anti-FLAG (Sigma), anti-ILF3 (Santa Cruz), anti- FMR1 (Santa Cruz), anti-HA (Roche), anti-Dicer1 (Santa Cruz), anti-AGO2 (Santa Cruz) and anti-BAG5 (Abcam).

Example 11: siRNA-Mediated Down-Regulation of Human Stau1

232. HeLa cells (5×10^6) were grown in DMEM medium (Gibco-BRL) containing 10% fetal bovine serum (Gibco-BRL) in 100-mm dishes and transiently transfected with 50 nM of *in vitro*-synthesized small interfering (si)RNA (Dharmacon) using Oligofectamine Reagent (Invitrogen). Stau1 was down-regulated using 5'-r(CCUAUAACUACAACAUGAG)d(TT)-3' (SEQ ID NO. 84). Protein was purified from half of the cells using passive lysis buffer (Promega) and used to determine the extent of down-regulation. RNA was purified from the other half using TRIzol Reagent (Invitrogen), 72 h after siRNA introduction, and analyzed using microarrays. Stau1(A) siRNA consisted of 5'-r(GUUUGAGAUUGCACUAAA)d(TT)-3' (SEQ ID NO. 85), and Upf1(A) siRNA consisted of 5'-r(AACGUUUGCCGUGGAUGAG)d(TT)-3' (SEQ ID NO. 86).

Example 12: Tethering Experiments

233. HeLa cells (2×10^6) were transiently transfected using Lipofectamine Plus (Invitrogen) with 0.3 μ g of the reporter plasmid pcFLuc or pcFLuc-MS2bs, 0.02 μ g of the reference plasmid pRLuc, and 5 μ g of one of the following effector plasmids: pMS2-HA, pMS2-HA-Stau1, pcNMS2, pcNMS2-Upf1, pcNMS2-Upf2 or pcNMS2-Upf3. Cells were harvested two days later. Protein was purified from half of the cells using passive lysis buffer (Promega), and total RNA was purified from the other half using TRIzol Reagent (Invitrogen).

Example 13: RT-PCR

234. FLuc-MS2bs mRNA or FLuc(UAA $\rightarrow$ CAA)-MS2bs mRNA was amplified using the primers 5'-CAACACCCCAACATCTTCG-3' (SEQ ID NO. 87) (sense) and 5'-CTTTCCGCCCTTCTTGGCC-3' (SEQ ID NO. 88) (antisense). GI-MS2bs or GI(UAA $\rightarrow$ UAC)-MS2bs was amplified using the primers 5'-AATACGACTCACTATAGGGA-3' (SEQ ID NO. 89) (sense), which anneals to the T7 promoter, and 5'-GATACTTGTGGGCCAGGGCA-3' (SEQ ID NO. 90) (antisense). FLuc-Arf1 mRNA was amplified using the same T7 promoter primer (sense) and 5'-TCTAGAGGATAGAATGGCG-3' (SEQ ID NO. 91) (antisense). PAICS mRNA was amplified using the primers 5'-AGCAGGCTGGTACCGTCCG-3' (SEQ ID NO. 92) (sense) and 5'-ACCAATGTTTCAGTACCTCAG-3' (SEQ ID NO. 93) (antisense).

Example 14: Microarray analyses

235. HeLa-cell RNA was purified using TriZol reagent (Invitrogen) and deemed to be intact using an RNA 6000 Nano LabChip® (Agilent) together with a Bioanalyser 2100 and Biosizing software (Agilent). Biotin-labeled cRNAs were generated and hybridized to U133 Plus 2.0 Array human gene chips (comprising more than 47,000 transcripts and variants). Hybridized chips were scanned using an Affymetrix GENECHIP® 3000 Scanner. Results were recorded using the GENECHIP® Operating Software (GCOS) platform, which included the GENECHIP® Scanner 3000 high-resolution scanning patch that enables feature extraction (Affymetrix). Notably, the Affymetrix Gene Expression Assay identifies changes that are greater than 2-fold with 98% accuracy. Arrays were undertaken using three independently generated RNA samples. Transcripts that showed at least a 1.8-fold increase in abundance with a p value of less than 0.05 in each of the three analyses were scored as potential SMD targets.

236. **Example 15: RNA purification, poly(A)+ RNA preparation, and RT coupled to either semiquantitative (sq) or realtime (q)PCR.**

RNA was purified from total, nuclear or cytoplasmic HeLa-cell fractions or immunoprecipitated from total-cell lysates using TRIzol (Invitrogen). Poly(A)+ RNA was

extracted from total-cell RNA using the Oligotex mRNA Mini Kit (Qiagen). Alternatively, RNA derived from different human tissues (Ambion). RT-sqPCR and RT-qPCR were performed as described¹ using the designated primer pairs (Table 11).

5 Table 11

RNA	Sense primer	Antisense primer
½-sbsRNA1	5'-CAGCATTCTGAGTAGCTGGGATC-3' (SEQ ID NO: 94)	5'-CAGTTGTAAGCATATTTGGGTTAC-3' (SEQ ID NO: 121)
Primer #1 (Fig. 5c)	5'-CAGCATTCTGAGTAGCTGGGATC-3' (SEQ ID NO: 95)	
#2	5'-CCTTCCAACCTCAGCATTCTGAG-3' (SEQ ID NO: 96)	
#3	5'-GCTCAAGCAATCCTTCCAACCTC-3' (SEQ ID NO: 97)	
#4	5'-TCTTTTAACTCCTGGGCTCAAGC-3' (SEQ ID NO: 98)	
#5	5'-GGCTGGAATACAGTGGTGTGATC-3' (SEQ ID NO: 99)	
#6	5'-CAGTCTCACTCTGTTGCCCAGGC-3' (SEQ ID NO: 100)	
#7	5'-AAAGGAGAGACAGTCTCACTCTG-3' (SEQ ID NO: 101)	
#8	5'-AGGACAATGACCCAAGAAGTAGG-3' (SEQ ID NO: 102)	
#9	5'-CCTTTGACAAAGGACAATGACCC-3' (SEQ ID NO: 103)	
#10	5'-GGACTTCCTCTGGCATGTAGG-3' (SEQ ID NO: 104)	
SMG7 mRNA	5'-CCAAAGGAGACCATCTGACC-3' (SEQ ID NO: 105)	5'-TTCACCTCATCTCGGCTTTCC-3' (SEQ ID NO: 122)
SERPINE1 mRNA	5'-ACCGCCAATCGCAAGGCACC-3' (SEQ ID NO: 106)	5'-GCTGATCTCATCCTTGTTC-3' (SEQ ID NO: 123)
FLJ21870 mRNA	5'-AACGACCTGTAAAGGCCACTC-3' (SEQ ID NO: 107)	5'-TCCTTACTTTGCAGGCATCCAG-3' (SEQ ID NO: 124)
FLUC mRNA	5'-AATACGACTCACTATAGGGA-3' (SEQ ID NO: 108)	5'-TCTAGAGGATAGAATGGCG-3' (SEQ ID NO: 125)
MUP mRNA	5'-CTGATGGGGCTCTATG-3' (SEQ ID NO: 109)	5'-TCCTGGTGAGAAGTCTCC-3' (SEQ ID NO: 126)
½-sbsRNA1 (Fig. 6a only)	5'-CAGCATTCTGAGTAGCTGGGATC-3' (SEQ ID NO: 110)	5'-GAACCTGTACCCTTCAGCTTACC-3' (SEQ ID NO: 127)
½-sbsRNA1 ^R (Fig. 6a only)	5'-CAGCATTCTGAGTAGCTGGGATC-3' (SEQ ID NO: 111)	5'-GAACCCATACAGTTCAGCTCAGA-3' (SEQ ID NO: 128)
IL7R mRNA	5'-AAGTGGCTATGCTCAAAATG-3' (SEQ ID NO: 112)	5'-TTCAGGCACTTTACCTCCAC-3' (SEQ ID NO: 129)
½-sbsRNA2	5'-GTCCAGGAAGAAGGTGCAAAG-3' (SEQ ID NO: 113)	5'-GGCTACCATATTGGACAGCAC-3' (SEQ ID NO: 130)

½-sbsRNA3	5'-CTAAATCTTCCCATGAAATAGGTG-3' (SEQ ID NO: 114)	5'-GGCCTTTGATAGAAATGTGTAGG-3' (SEQ ID NO: 131)
½-sbsRNA4	5'-GGCGTTTGTAGATCACTCCTTC-3' (SEQ ID NO: 115)	5'-ATGCGTGGTGAAACTCAACTGG-3' (SEQ ID NO: 132)
GAPDH mRNA	5'-AAGGTCGGAGTCAACGGATTTG-3' (SEQ ID NO: 116)	5'-ATGACAAGCTTCCCCGTTCTCAG-3' (SEQ ID NO: 133)
RAB11FIP1 mRNA	5'-CCTGCTGAAGACCTTGTGAGAAG-3' (SEQ ID NO: 117)	5'-AGGATGAAGTCGGGGCTTGAC-3' (SEQ ID NO: 134)
CDCP1 mRNA	5'-GCAAGGTCTACCTGAGGACC-3' (SEQ ID NO: 118)	5'-CAGTCAAGTCCACAGTCCTTGG-3' (SEQ ID NO: 135)
MTAP mRNA	5'-TCTTGTGCCAGAGGAGTGTG-3' (SEQ ID NO: 119)	5'-CCACCGAAACTGCTTCCTCG-3' (SEQ ID NO: 136)
BAG5 mRNA	5'-CCCAGTGGGAGTGCTTGTGAAA-3' (SEQ ID NO: 120)	5'-AAGACGTTCTGTCTCCTGTGCT-3' (SEQ ID NO: 137)

In a few experiments RT primed using oligo(dT)18 rather than random hexamers. RT-sqPCR analyses situated under wedges in the leftmost lanes of figures utilized 2-fold dilutions of RNA and show that data fall in the linear range. RT-PCR values include the standard deviation obtained in the specified number of independently performed experiments.

Example 16: RNase protection assay (RPA) and primer extension.

237. The RPA employed the RPA III RNase Protection Assay Kit (Ambion). Uniformly labeled RNA probes (107 cpm/μg) were generated by transcribing linearized pcDNA3.1(+)/Zeo_Chrl1_66193000-66191383 *in vitro* using α-[P32]-UTP (Perkin Elmer) and the MAXIscript Kit (Ambion). Each probe (105 cpm) was incubated with poly(A)+ HeLa-cell RNA (10 μg) or yeast RNA (10 μg) in hybridization buffer (Ambion) at 42°C overnight and subsequently cleaved using RNase A and RNase T1 (Ambion; 1:200) at 37°C for 30 min. Input probe (1/1000) and cleaved products were resolved in a 3.5% polyacrylamide-denaturing gel and visualized using a Typhoon Phosphorimager (GE Healthcare).

238. Primer extension was performed using poly(A)+ HeLa-cell RNA (10 μg), Superscript II (Invitrogen) and the ½-sbsRNA1-specific antisense primer 5'-GAGTTAAAAGAGGCTGCAGTG-3' (SEQ ID NO: 138). DNA sequencing was executed using the SILVER SEQUENCING DNA Sequencing System (Promega), the same antisense primer and pcDNA3.1(+)/Zeo_Chrl1_66193000-66191383. Primer extension and sequencing products were resolved in an 8% polyacrylamide-denaturing gel and visualized using a Typhoon Phosphorimager.

Example 17: Fluorescence and phase-contrast microscopy.

239. Cells were visualized using a Nikon Eclipse TE2000-U inverted fluorescence microscope and, for phase microscopy, a 480-nm excitation spectra. Images were captured utilizing TILLVISION software (TILL Photonics).

240. Throughout this application, various publications are referenced. The disclosures of these publications in their entirety are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains. The references disclosed are also individually and specifically incorporated by reference herein for the material contained in them that is discussed in the sentence in which the reference is relied upon.

241. It will be apparent to those skilled in the art that various modifications and variations can be made in the present invention without departing from the scope or spirit of the invention. Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

Example 18: STAU in All Classes of Vertebrates Shares a Conserved Motif N-Terminal to ‘RBD’5

242. Using yeast two-hybrid analyses, full-length human (h)STAU1 was shown to interact with amino acids 408-496 of hSTAU1⁵⁵. These amino acids consist of the C-terminus of hSTAU1⁵⁵ and include ‘RBD’5 (Figure 10A), which is referred to as ‘RBD’5 considering it has only 18% sequence identity to the prototypical hSTAU1 RBD3 and, more to the point, fails to bind dsRNA. Using Clustal W, multiple sequence alignments of full-length hSTAU1 with hSTAU2 and STAU homologs in different species, including representatives of the five major vertebrate classes (mammals, reptiles, amphibians, birds, and fish), revealed a conserved sequence residing N-terminal to ‘RBD’5 that consists of amino acids 371-390 of hSTAU1⁵⁵ (Figure 10B). This motif is called the Staufen-swapping motif (SSM; Figure 10B) for reasons explained below. Despite an identifiable ‘RBD’5, SSM is absent from D. melanogaster STAU as well as some invertebrates such as *Caenorabditis elegans* (Figure 11). However, STAU in other invertebrates, including the acorn worm *Saccoglossus kowalevskii* and the deer tick *Ixodes capularis*, contain both ‘RBD’5 and SSM (Figure 11).

243. SSM is interesting given its proximity to the TBD, which spans amino acids 282-372 (Figure 10A), and its overlap with the region that recruits hUPF1 during SMD, which spans amino acids 272-405. Sequence alignments also demonstrated that, compared to

hSTAU1 'RBD'5, 'RBD'5 of all hSTAU2 isoforms lacks the predicted β -sheet and the N-terminus of α 2 (Figure 10B).

Example 19: SSM-'RBD'5 Forms a Homodimer in Solution and in Human Cells

244. Since sequence conservation generally implies function, and considering that
5 dimerization required that one of the interacting molecules consist of full-length hSTAU1,
whether SSM-'RBD'5, rather than 'RBD'5 alone, forms a homodimer was tested. The use of
SSM-'RBD'5 was further justified because a search for a region of hSTAU1⁵⁵ that includes
'RBD'5, is stable in solution, and is suitable for crystallographic studies revealed that amino
acids 367-476, i.e., SSM-'RBD'5, gave the best Xtal Pred score, although the score was below
10 suboptimal. This is largely because 'RBD'5 has a higher instability index than does SSM-
'RBD'5. Thus, at least theoretically, SSM provides stability to 'RBD'5.

245. After purifying GST-hSTAU1⁵⁵(367-476) from *E. coli* and removing the GST
tag, SSM-'RBD'5 migrated during gel filtration at the size of a dimer. Sedimentation velocity
determinations using analytical ultracentrifugation confirmed that the average weight
15 distribution of SSM-'RBD'5 shifted to lower Svedberg values at lower concentrations (Figure
10C). The best-fit model for SSM-'RBD'5 (0.0090 mg/ml root mean standard deviation (rmsd)
with 95% confidence limits) was one of rapid monomer ($1.32 \pm 0.02/-0.03$ S)–dimer ($2.21 \pm$
0.01 S) equilibrium where the dimer K_d was 79 ± 9 μ M.

246. To obtain a physiologically relevant assay, 'RBD'5 was tested to determine if it
20 is sufficient for SSM-'RBD'5 homodimer formation in human cells. 'RBD'5 was expressed
with an N-terminal tag since experiments using *E. coli*-produced 'RBD'5 revealed that an N-
terminal GST tag or SSM moiety was required for stability and/or solubility in vitro. Human
embryonic kidney (HEK)293T cells were transiently transfected with a mixture of two
plasmids: (i) pEGFP-'RBD'5, which produces monomeric enhanced green fluorescence protein
25 (EGFP)-'RBD'5 and either pmRFP-SSM-'RBD'5 or pmRFP-'RBD'5, which produce
monomeric red fluorescence protein (mRFP)-SSM-'RBD'5 or mRFP-'RBD'5, respectively; or
(ii) pEGFP-SSM-'RBD'5 and either pmRFP-SSM-'RBD'5 or pmRFP-'RBD'5 (Figure 1D,
upper). Notably, EGFP and mRFP harbor mutations that disrupt dimer formation that interferes
with the analyses. Cell lysates were immunoprecipitated in the presence of RNase A using anti-
30 GFP or, to control for nonspecific immunoprecipitation (IP), mouse(m)IgG.

247. Western blotting demonstrated that both EGFP-tagged proteins were expressed
at comparable levels prior to IP, as were both mRFP-tagged proteins (Figure 10D, lower).
Furthermore, anti-GFP immunoprecipitated comparable amounts of each EGFP-tagged protein

but did not immunoprecipitate calnexin, and mIgG failed to detectably immunoprecipitate any protein (Figure 10D, lower). EGFP-SSM-‘RBD’5 co-immunoprecipitated with mRFP-SSM-‘RBD’5 and mRFP-‘RBD’5 with comparable efficiencies, whereas EGFP-‘RBD’5 co-immunoprecipitated with mRFP-SSM-‘RBD’5 but not mRFP-‘RBD’5 (Figure 10D, lower).

Both EGFP-‘RBD’5 and EGFP-SSM-‘RBD’5 also co-immunoprecipitated with endogenous hSTAU1 isoforms (Figure 10D, lower), consistent with the finding that ‘RBD’5 alone can interact with full-length hSTAU1 in yeast two-hybrid analyses. hSTAU1 ‘RBD’5-‘RBD’5 dimerization cannot occur between two proteins that each contain only ‘RBD’5 but can occur if one of two ‘RBD’5-containing molecules contributes an SSM.

10 Example 20: Structure of hSTAU1 SSM-‘RBD’5

248. To understand the atomic details of this dimerization interaction, the X-ray crystal structure of SSM-‘RBD’5 at 1.7 Å resolution was determined (Table 12).

Table 12: Top matches to STAU1 ‘RBD’5 identified by Dali.

PDB-Chain	Dali Z-score	Cα rmsd (Å)	% ID	Protein	Organism	Binds dsRNA?	Bound to dsRNA in this structure?
*3LLH-B/A	*10.8/10.7	*1.3/1.1	*19/20	TARBP2 RBD1	Homo sapiens	Yes	No
*2NUE-A/B	*10.6/10.6	*2.0/1.8	*22/22	RNase III	Aquifex aeolicus	Yes	Yes
1YYK-A	10.5	2.0	22	RNase III	Aquifex aeolicus	Yes	Yes
1DI2-A	10.5	1.5	22	Xlrbpa RBD2	Xenopus laevis	Yes	Yes
2NUG-B	10.5	1.9	22	RNase III	Aquifex aeolicus	Yes	Yes

15

249. Results indicated that ‘RBD’5 does adopt the α - β - β - α topology of a prototypical RBD (Figure 12A). One molecule is present per asymmetric unit, indicating that any existing dimer in the crystal structure is derived from crystallographic symmetry mates.

20 250. SSM, which directly binds ‘RBD’5 (Figure 12A,B), forms two α -helices (SSM α 1 and α 2) that are connected by a tight turn. These helices form a ‘V’-shape that straddles ‘RBD’5 α 1 (Figure 12A,B). ‘RBD’5 α 1 and α 2 pack behind the β -sheet that connects the two helices in the primary sequence (Figure 12A). The ‘V’-shape created by the ‘RBD’5 helices in turn straddles SSM α 1 (Figure 12A,B).

25 251. The interactions between SSM and ‘RBD’5 form a core composed of residues with hydrophobic side chains (Figure 12B). The external solvent boundary of this core is

defined by T371 of the longer of the two SSM α -helices, $\alpha 1$; the hydrophobic methyl group of T371 inserts into the hydrophobic core, and the T371 hydroxyl group points outward (Figure 12B). I374, A375, L378 and L379 of SSM $\alpha 1$ also contribute to the hydrophobic core as do A387 of SSM $\alpha 2$ and 'RBD'5 $\alpha 1$ constituents P408 (which starts $\alpha 1$), L412, the ring of Y414, L415, and the β and γ carbons of the Q419 (which ends $\alpha 1$) (Figure 12B). Additionally, 'RBD'5 $\alpha 2$ contributes A465, L469 and L472 (Figure 12B). Of the 'RBD'5 hydrophobic core-contributing and SSM-interacting residues, P408 is absent from nearly all STAU proteins that lack an SSM. However, regardless of the presence of an SSM, L412, L415 and A465 are invariant, and Y414, L469 and L472 are generally conserved (Figure 11).

252. Two polar interactions exist at the interface between SSM and 'RBD'5 and appear to position SSM and 'RBD'5 relative to one another to facilitate what is largely a hydrophobic-driven interaction. In the first, the side-chain hydroxyl group of SSM T371 and the main-chain oxygen of K367 hydrogen bond with the amine group of 'RBD'5 Q419, while the ϵ -amine of K567 interacts with the hydroxyl group of Q419 (Figure 12B). All vertebrate STAU proteins contain a residue having similar character and position to T371 e.g., Ser replaces Thr in human and mouse STAU2 proteins (Figure 11), and Q419 is strictly conserved. The second polar interaction involves a strongly conserved basic charge (in all vertebrate STAU proteins examined except *D. rerio* STAU, where the residue is Asn; Figure 10B) contributed by SSM R376 in hSTAU1 that interacts with a citrate ligand present in the crystal structure; otherwise, R376 interacts with the main-chain oxygen of E474 near the C-terminus of 'RBD'5 $\alpha 2$, which is also invariant in vertebrate STAU homologs (Figure 10B).

253. Interestingly, residues without strict conservation within SSM, i.e., M373, Y380, G381, T383 and P385, are positioned on its solvent-exposed side, opposite to the interface that interacts with 'RBD'5. This indicates that the conservation of SSM residues has been driven by a requirement to interact with 'RBD'5. Furthermore, 'RBD'5 Q419 and G420, the latter of which is most likely needed to end $\alpha 1$ and supply flexibility to the T371:Q419 interaction, are strictly conserved in all SSM-containing STAU proteins that were examined, whereas no STAU protein that lacks an SSM contained either of these residues (Figure 11). These findings indicate that SSM and 'RBD'5 have co-evolved to interact with one another either in cis (i.e., when STAU is a monomer), in trans (i.e., when STAU is a dimer), or both.

Example 21: SSM and 'RBD'5 are Domain-Swapped Between Two Symmetry-Related Molecules in the Crystal Structure

254. There is little-to-no electron density in the X-ray crystal structure for amino acids 397-404 in the loop that connects SSM and 'RBD'5 (Figures 12A). This high level of disorder led to difficulties in interpreting whether SSM is bound in cis to its own 'RBD'5 or RBD'5 derives from an adjacent molecule in the crystal lattice, i.e., is the consequence of domain-swapping between two molecules.

255. Assuming the cis-configuration, proximal molecules in the crystal lattice were examined using PISA (Figure 13A,B) to define the dimer interface. The largest symmetric crystal-packing interface, which was only $\sim 764 \text{ \AA}^2$ (from each molecule), was formed between the 'RBD'5s of adjacent molecules (Figure 13A,B) and was defined by a PISA complexation significance score of zero (i.e., the least significant), indicating it is not biologically relevant. Furthermore, SSM-'RBD'5(E424H, D427V), which contains mutations at this interface (Figure 13B), produced a gel-filtration chromatogram identical to that of SSM-'RBD'5(WT) (Figure 13C), i.e., dimers and not monomers were observed.

256. A perfect CSS score of 1.0 was obtained for the trans configuration (Figure 13A), in which a highly conserved SSM of one molecule binds to the 'RBD'5 of a second molecule (Figure 12B) to create a 38-residue surface area of $\sim 1249 \text{ \AA}^2$ for each molecule (Figure 13A). Possibly, the interaction occurs in cis when hSTAU1 is a monomer and in trans when it is a dimer.

Example 22: Comparison of 'RBD'5 to an RBD that Binds dsRNA

257. The structural elements that render 'RBD'5 unable to bind dsRNA were addressed. Remarkably, the three RBD structures that according to the Dali server are most similar to 'RBD'5 do bind dsRNA (Table 12). These structures are RBD1 of human TAR RNA-binding protein 2 (hTRBP2; PDB ID 3LLH; Z-score = 10.8; rmsd = 1.3 $\text{\AA}$), the RBD of RNase III from the thermophilic prokaryote Aquifexaerophilus (PDB ID 2NUE; Z-score = 10.6; rmsd = 2.0 $\text{\AA}$), and RBD2 of Xenopus laevis RNA-binding protein A (Xlrpba; PDB ID 1DI2; Z-score = 10.5; rmsd = 1.5 $\text{\AA}$).

258. hTRBP2 RBD1 is the most similar to hSTAU1 'RBD'5. To address how STAU1 'RBD'5 adopt a fold similar to that of hTRBP2 RBD1 yet fail to bind RNA, the two structures were superimposed (Figure 14A) to create a structure-based sequence alignment (Figure 14B). While the two structures are nearly identical, hSTAU1 'RBD'5 has a shorter loop 2 (L2) and a longer L3 compared to hTRBP2 RBD1. Furthermore, none of the key residues that typify the three RNA-binding regions of canonical RBDs and are present in hTRBP2 RBD1 are found in hSTAU1 'RBD'5. The most apparent differences reside in regions 2 and 3. L2, which

is shortened and is unable to reach the minor groove of dsRNA, lacks a His residue that in hTRBP2 RBD1 interacts with the dsRNA minor groove (Figure 14A). In hSTAU1 'RBD'5 region 3, the positively charged residues of hTRBP2 RBD1 that interact with the negatively charged phosphate backbone spanning the dsRNA major groove are negatively charged and can actually repel dsRNA (Figure 14A, C, and D). Consistent with this view, *D. melanogaster* STAU RBD3 and hTRBP2 RBD1 maintain a basic charge in region 3 despite having differences in the lengths of L4 and $\alpha 2$. In fact, *D. melanogaster* STAU RBD3 RNA binding was lost when either of the first two conserved Lys residues (K59 and K60 in hTRBP2 RBD1; Figure 14A) of region 3 were mutated to alanine. As inferred from the NMR structure of *D. melanogaster* STAU RBD3, hSTAU1 RBD3 likewise maintains a basic charge in region 3. The absence of the conserved His residue or a functional equivalent in hSTAU1 'RBD'5 L2 of region 2 contributes to the loss of dsRNA binding as evidenced by the finding that *D. melanogaster* STAU RBD3 RNA binding was lost when this L2 His was changed to Ala.

Example 23: SSM-'RBD'5 Domain-Swapping Facilitates the Interaction Between hSTAU1-hSTAU1 as well as hSTAU1-hSTAU2, Where 'RBD'5 $\alpha 1$ is Key

259. From the structure, the two α -helices of 'RBD'5 interact with the two α -helices of SSM. However, the finding that hSTAU1⁵⁵ can interact with all hSTAU2 isoforms, the smallest of which (hSTAU2⁵²) has a C-terminus that ends five amino acids after 'RBD'5 $\alpha 1$ (Figure 10B), indicates that $\alpha 1$ must constitute the minimal region of 'RBD'5 necessary to interact with SSM.

260. To test this idea, HEK293T cells were transiently transfected with hSTAU1⁵⁵-HA₃ and one of three siRNA-resistant (R) plasmids that produce either hSTAU1^{55(R)}-FLAG, hSTAU1^{55(R)} Δ (C-Term)-FLAG or hSTAU1^{55(R)} Δ (SSM-'RBD'5)-FLAG, each of which was expressed at the level of cellular hSTAU1. hSTAU1^{55(R)} Δ (C-Term)-FLAG lacks all amino acids C-terminal to 'RBD'5 $\alpha 1$. Cell lysates were immunoprecipitated in the presence of RNaseA using anti-FLAG or, as a negative control, mIgG.

261. Western blotting demonstrated that the three FLAG-tagged proteins were expressed at comparable levels prior to IP, were immunoprecipitated with comparable efficiencies using anti-FLAG, and were not immunoprecipitated with mIgG. The level of hSTAU1⁵⁵-HA₃ or cellular hUPF1 that co-immunoprecipitated with hSTAU1^{55(R)} Δ (SSM-'RBD'5)-FLAG was only ~10% the level of, respectively, hSTAU1⁵⁵-HA₃ or cellular hUPF1 that co-immunoprecipitated with hSTAU1^{55(R)}-FLAG or hSTAU1^{55(R)} Δ (C-Term)-FLAG. IPs of the same transfections using either anti-HA, or rIgG as a negative control, revealed that the

level of hSTAU1^{55(R)}Δ(SSM-‘RBD’5)-FLAG that co-immunoprecipitated with hSTAU1⁵⁵-HA was only ~10% the level hSTAU1^{55(R)}-FLAG or hSTAU1^{55(R)}Δ(C-Term)-FLAG that co-immunoprecipitated with hSTAU1⁵⁵-HA₃. These results indicate that domain-swapping between SSM and ‘RBD’5 is the major determinant of hSTAU1 homodimerization and that

5 ‘RBD’5 α1 is necessary and sufficient to interact with SSM.

262. Since hSTAU1 recruits hUPF1 to SMD targets during SMD, cellular hUPF1 was also assayed. Remarkably, the co-IP of hUPF1 with each hSTAU1^{55(R)}-FLAG variant correlated with homodimerization ability. Consistent with this, the co-IP of hUPF1 with hSTAU1⁵⁵-HA₃ did not vary among transfections (Figure 4B, lower). Assays of the three

10 detectable cellular hSTAU2 isoforms demonstrated that hSTAU2 co-immunoprecipitates with each hSTAU1^{55(R)}-FLAG variant with the same relative efficiency as did hSTAU1⁵⁵-HA₃.

263. Since hSTAU1^{55(R)}Δ(SSM-‘RBD’5)-FLAG still had residual dimerization activity (10% that of hSTAU1^{55(R)}-FLAG), and in view of reports that hSTAU1 ‘RBD’2 amino acids 37-79 can interact with full-length hSTAU1, the ability of E. coli-produced ‘RBD’2-

15 RBD3 amino acids 43-173 to dimerize was assayed. Gel filtration demonstrated that ‘RBD’2-RBD3 indeed migrated at the position expected of an ‘RBD’2-RBD3-‘RBD’2-RBD3 dimer.

Example 24: Domain-Swapping of SSM and ‘RBD’5 α1 is Important for SMD

264. To test the importance of hSTAU1⁵⁵ dimerization to SMD, HEK293T cells were transiently transfected with: (i) STAU1(A)siRNA; (ii) plasmid expressing one of the three

20 hSTAU1^{55(R)}-FLAG variants or, as a control, no protein; (iii) three plasmids that produce a firefly luciferase (FLUC) reporter mRNA, i.e., FLUC-No SBS mRNA, which lacks an SBS, FLUC-hARF1 SBS mRNA, which contains the hARF1 SBS, and FLUC-hSERPINE1 3’ UTR, which contains the 3’ UTR and, thus SBS of mRNA encoding serpin peptidase inhibitor clade e, member 1; and (iv) a reference plasmid that produces renilla luciferase (RLUC) mRNA. In

25 parallel, cells were transfected with (i) Control siRNA, (ii) plasmid producing no hSTAU1^{55(R)}-FLAG protein, (iii) the three FLUC reporter plasmid DNAs, and (iv) the RLUC reference plasmid.

265. Western blotting of cell lysates demonstrated that STAU1(A)siRNA reduced the abundance of cellular hSTAU1 to ~10 % the level in Control siRNA-treated cells and that

30 each hSTAU1^{55(R)}-FLAG variant was expressed at comparable abundance. When semi-quantitative RT-PCR was used to normalize the level of each FLUC mRNA to the level of RLUC mRNA, which controls for variations in transfection efficiencies and RNA recovery, the normalized level of FLUC-No SBS mRNA, which is not an SMD target, was found to be

essentially identical in all transfections, as expected. In contrast, the normalized level of FLUC-hARF1 SBS mRNA or FLUC-hSERPINE1 3' UTR mRNA was increased ~2-fold in the presence of STAU1(A) siRNA alone, consistent with an inhibition of SMD. This inhibition was reversed by 50% when hSTAU1^{55(R)}-FLAG or hSTAU1^{55(R)}Δ(C-Term)-FLAG were
 5 expressed but not when hSTAU1^{55(R)}Δ(SSM-‘RBD’5)-FLAG was expressed. Thus, hSTAU1^{55(R)}-FLAG and hSTAU1^{55(R)}Δ(C-Term)-FLAG can functionally compensate for the siRNA-mediated down-regulation of cellular hSTAU1 more efficiently than can hSTAU1^{55(R)}Δ(SSM-‘RBD’5)-FLAG.

266. hSTAU1 dimerization is important for SMD, because hSTAU1 dimers bind an
 10 SBS and/or UPF1 more efficiently than hSTAU1 monomers, but also because hSTAU1^{55(R)}Δ(SSM-‘RBD’5)-FLAG can bind hUPF1 inefficiently. Considering that deleting the TBD and adjacent SSM of hSTAU1 ablated hUPF1 binding to an essentially undetectable level, and that ‘RBD’5 alone does not bind hUPF1 (Figure 10D), whether inhibiting hSTAU1 dimerization by expressing ‘RBD’5 inhibited hSTAU1 binding to hUPF1 was tested. To this
 15 end, HEK293T cells were transiently transfected with plasmid producing hSTAU1^{55(R)}-FLAG, hSTAU1⁵⁵-HA₃, and either mRFP-‘RBD’5 (from pmRFP-‘RBD5’) or no protein (from pmRFP). Lysates were immunoprecipitated in the presence of RNase A using anti-FLAG or, as a negative control, mIgG.

267. Western blotting demonstrated that comparable amounts of hSTAU1^{55(R)}-FLAG
 20 were expressed and immunoprecipitated using anti-FLAG in the presence or absence of ‘RBD’5, and hSTAU1^{55(R)}-FLAG and hSTAU1⁵⁵-HA₃ were each expressed at a level comparable to that of cellular hSTAU1. ‘RBD’5 expression reduced the amount of hSTAU1⁵⁵-HA₃ that co-immunoprecipitated with hSTAU1^{55(R)}-FLAG to 35-40% of the amount that co-immunoprecipitated in the absence of ‘RBD’5 expression. The finding that ‘RBD’5 expression
 25 reduced the amount of cellular hUPF1 that co-immunoprecipitated with hSTAU1^{55(R)}-FLAG also to 35-40% of the amount that co-immunoprecipitated in the absence of ‘RBD’5 expression indicates that hUPF1 binds hSTAU1 dimers more efficiently than it binds hSTAU1 monomers.

Example 25: hSTAU1 Homodimerization Occurs Through the SSM

268. The hSTAU1 SSM, which is a two-helix motif that interacts with the dsRNA-
 30 binding-deficient ‘RBD’5 of another hSTAU1 molecule. A complete SSM and α1 of ‘RBD’5, which are required for this interaction, can be found in the STAU proteins of all vertebrate classes. The majority of the ‘RBD’5 hydrophobic residues that contribute to SSM-‘RBD’5 homodimerization via domain-swapping are generally present in other RBDs. In particular, that

P408, L412, A465, L469 and L472 are conserved between hSTAU1 'RBD'5 and hTRBP2 RBD1 (Figure 14B) most likely explains why hSTAU1 'RBD'5 and hTRBP2 RBD1 can be structurally superimposed (Figure 14A). However, hSTAU1 'RBD'5 residues Y414 and L415, which are positioned, respectively, in or proximal to dsRNA-interacting region 1, are not conserved in other RBDs (Figure 14B). SSM is a modular adaptation in many if not all vertebrate STAU homologs that, through its interaction with 'RBD'5, adds greater functionality to full-length protein, i.e., the potential for STAU to dimerize if not multimerize considering the 'RBD'2-'RBD'2 interaction.

Example 26: hSTAU1 Homodimerization Contributes to SMD

269. Compared to hSTAU1 monomers, hSTAU1 dimers bind hUPF1 more efficiently and mediate SMD more effectively. Thus, cells can regulate SMD by controlling hSTAU1 dimer formation. Since homodimers of SSM-'RBD'5 are less stable ($K_d = 79 \mu\text{M}$; see also Figure 10C) than are complexes of full-length hSTAU1 and dsRNA ($K_d = 10^{-9} \text{ M}$), hSTAU1 binding to dsRNA can nucleate hSTAU1 dimerization. Proteins known to dimerize on double-stranded nucleic acid are exemplified by transcriptional activators, the adenosine deaminases ADAR1 and ADAR2, and protein kinase RNA-activated (PKR), where binding to viral dsRNA promotes PKR phosphorylation activity by allowing dimerization of the kinase domain.

Example 27: 'RBD'5 has Diverged from a True RBD to Serve a New Function

270. Assuming 'RBD'5 evolved from a functional RBD, it not only lost the ability to bind dsRNA but gained the ability to interact with SSM. The structure together with models that compare 'RBD'5 to true RBDs, such as hTRBD2 RBD1 and RBD2 in association with dsRNA, clearly illustrate that RBD regions 2 and 3, which interact with the minor groove and bridge the proximal major groove of dsRNA, respectively, are altered in 'RBD'5 (Figure 14D). The role of region 1 in $\alpha 1$, at least for true RBDs, determines RNA recognition specificity, where $\alpha 1$ can bind the major groove or possibly distinguishing features such as loops at the apex of dsRNA. In 'RBD'5, $\alpha 1$ specifies SSM recognition, although 'RBD'5 $\alpha 1$ interacts with SSM using a face that is orthogonal to the face that interacts with dsRNA in a true RBD. The data indicate that 'RBD'5 $\alpha 1$ is sufficient for hSTAU1 dimerization and, together with four L1 amino acids, is the only region of 'RBD'5 that exists in the smallest STAU2 isoform (Figure 10B).

271. Interestingly, what remains of 'RBD'5 in the hSTAU2 isoforms with the longest C-termini corresponds to the regions of hSTAU1 'RBD'5 that contact SSM directly,

i.e., 'RBD'5 α 1 and the C-terminus of 'RBD'5 α 2 (Figure 10B; 12B). Due to the lack of a sufficiently sized loop residing between hSTAU2 'RBD'5 α 1 and the remains of 'RBD'5 α 2, details of the dimerization interaction between hSTAU2-hSTAU2 and hSTAU1-hSTAU1 can differ. However, residues of hSTAU2 'RBD'5 residing C-terminal to α 1 are dispensable for the SSM-mediated dimerization of hSTAU2, since they are dispensable for the SSM-mediated dimerization of hSTAU1.

Exmple 28: The RBD Fold as a Platform for New Functions

272. As reported here, the combination of a modified RBD, e.g., hSTAU1 'RBD'5, within the context of an adapter region, e.g., hSTAU1 SSM, can promote greater functionality within the larger, often modular and flexible framework of proteins that contain one or more RBDs that bind dsRNA. In support of this view, modifications that consist of anL1 Cys and anL3 His within the RBD of the Schizosaccharomyces pombe Dicer DCR1 protein work together with a 33-amino acid region that resides C-terminal to the RBD to form a zinc-coordination motif that is required for nuclear retention. Since this RBD binds dsRNA and dsDNA, these and other changes can additionally contribute to dsDNA binding.

273. 'RBD's that fail to bind dsRNA can acquire new functions independently of adjacent regions. As one example of this, 'RBD'5 region of Drosophila Staufen has adapted to bind the Miranda protein required for proper localization of prospero mRNA. As another example, hTRBP2 contains three RBDs, and the C-terminal RBD binds Dicer instead of dsRNA. Additionally, in contrast to the SSM-mediated dimerization of hSTAU1 'RBD'5, 'RBD'3 of Xlr bpa and its human homolog, p53-associated cellular protein PACT, appear to homodimerize independent of an accessory region.

Example 29: Sequence Alignments

274. Sequences were obtained from NCBI. Multiple protein sequence alignments were performed using Clustal W within BioEdit, which was used to generate figures.

Example 30: Protein Crystallization and Structure Determinations

275. Native crystal datasets were obtained using crystals produced from gel-filtration-purified hSTAU1 SSM-'RBD'5 and either the hanging-drop method for remote data collection at the Stanford Synchrotron Radiation Lightsource (SSRL) or the sitting-drop method for data collection at the Cornell High Energy Synchrotron Source (CHESS) (Table 12). An initial model was built using low-resolution experimental phases obtained from in-house single anomalous dispersion phases from an ethyl mercuric phosphate-soaked crystal. These coordinates were used for molecular-replacement and refined against a 2.2 Å native

dataset (Table 12). The final model was refined against a subsequent, 1.7 Å native data set. A consistent set of reflections for the free R-factor was maintained throughout. Structure figures were generated using PyMOL (Schrödinger, LLC). See Extended Experimental Procedures for crystallization and structure determination details.

5 **Example 31: HEK293T-Cell Transfections, and Protein and RNA Purification**

276. Human HEK293T cells were grown in Dulbecco's-modified eagle medium (Gibco-BRL) containing 10% fetal-bovine serum (Gibco-BRL). Cells were transiently transfected with plasmids using Lipofectamine 2000 (Invitrogen) or with siRNA using Oligofectamine (Invitrogen) as indicated in the figure legends. siRNAs consisted of hSTAU1
10 siRNA(A) and Negative Control #1 siRNA (Ambion). Protein was isolated using passive lysis buffer (Promega), and RNA was purified using TRIzol Reagent (Invitrogen).

Example 32: Plasmid Constructions

277. To generate pGEX-6p-1-hSTAU1-SSM-'RBD'5, the online servers MeDor (Lieutaud et al., 2008) and XtalPred (which gave an average score of 3) were used to guide the
15 choice of boundaries that include hSTAU1 SSM and 'RBD'5 and provide optimal protein stability and crystallization. The region of hSTAU1 cDNA that encodes amino acids 367-476 was PCR-amplified using pRSET-B hSTAU1 and the primer pair 5'-
AAAAGGATCCAAGGCCACGGTAACTGCCATG-3' (sense, where the BamHI site is underlined) (SEQ ID NO: 161) and 5'-
20 AAAAGAATTCTTATCAGTCCAACTCAGACAGCAAC-3' (antisense, where the EcoRI site is underlined) (SEQ ID NO: 162). The resulting PCR product was cleaved with BamHI and EcoRI and inserted into the BamHI and EcoRI sites of pGEX-6p-1 (GE Healthcare) to generate pGEX-6p-1-hSTAU1-SSM-'RBD'5. Notably, upon removing the GST-tag using PreScission Protease (GE Healthcare), the resulting protein contains the amino acids GPLGS
25 N-terminal to hSTAU1 amino acids 367-476.

278. To construct pGEX-6p-1-hSTAU1-SSM-'RBD'5(E424H, D427V), the QuikChangeTM method was used but with KOD DNA polymerase (Novagen), pGEX-6p-1-hSTAU1-SSM-'RBD'5 as template, and the primer pair 5'-
GATTCCAGGTTCAATACAAAGTCTTCCCCAAAAC-3' (sense) (SEQ ID NO: 163) and
30 5'-GTTTTTGGGGAAGACTTTGTAATGAACCTGGAATC-3' (antisense) (SEQ ID NO: 164).

279. To construct pEGFP-hSTAU1-SSM-'RBD'5 or pEGFP-hSTAU1-'RBD'5, pRSET B-STAU1 was PCR-amplified using 5'-AAAAGAATTC TTATCA

GTCCAACTCAGACAGCAAC-3' (antisense, EcoRI site is underlined) (SEQ ID NO: 165) and, respectively, 5'-AAAAAAGCTTAAGGCCACGGTAACTGCCATG-3' (sense, HindIII site is underlined) (SEQ ID NO: 166) or 5'-

AAAAAAGCTTAGACCCTCTGAGCAACTGGAC-3' (sense, HindIII site is underlined)

5 (SEQ ID NO: 167). PCR products were cleaved using EcoRI and HindIII and inserted into pEGFP-C3 (BD Biosciences Clontech).

280. To construct pmRFP-hSTAU1-SSM-'RBD'5 or pmRFP-hSTAU1-RBD'5, pRSETB-STAU1 was PCR-amplified using the same antisense primer that was used to amplify the pEGFP plasmids and, respectively, 5'-

10 AAAAAAGCTTCTAAGGCCACGGTAACTGCCATG-3' (sense, HindIII site is underlined) (SEQ ID NO: 168) or 5'-AAAAAAGCTTCTAGACCCTCTGAGCAACTGGAC-3' (sense, HindIII site is underlined) (SEQ ID NO: 169). PCR products were cleaved using EcoRI and HindIII and inserted into pmRFP. pmRFP derives from pDsRed-Express-C1 (Clontech Laboratories, Inc.) and contains mutations that render DsRed (i.e. mRFP) monomeric.

15 281. STAU1(A) siRNA-resistant phSTAU1^{55(R)}-HA₃ was derived from the PCR-amplification of pcDNA3/RSV-hSTAU-HA₃ using two sets of primer pairs: 5'-

CACCACATTGGTGTGCACCTCCAAGCTTGG-3' (sense A) (SEQ ID NO: 170) and 5'-CCGcTTcAGGGCGATTTCGAACACTTGACTTATTTcAGATTT-3' (antisense A, where underlined nucleotides confer siRNA-resistance, and) (SEQ ID NO: 171), and 5'-

20 AAATCTGAAATAAGTCAAGTGTTcGAAATcGCCcCTGAAgCGG-3' (sense B, where underlined nucleotides confer siRNA-resistance, and the two lower case letters were ineffective in directing a change from the wild-type sequence) (SEQ ID NO: 172) and 5'-

GAAGGCACAGTCGAGGCTGATCAGCGAG-3' (antisense B) (SEQ ID NO: 173). The resulting two PCR products were mixed and PCR-amplified using the first sense A and

25 antisense B primers. This PCR product was cleaved with KpnI and XbaI and inserted into pcDNA3/RSV-hSTAU-HA₃

282. To generate pcI-neo-hSTAU1^{55(R)}-FLAG, pcI-neo-hSTAU1^{55(R)}Δ(C-Term)-FLAG or pcI-neo-hSTAU1^{55(R)}Δ(SSM-'RBD'5)-FLAG, phSTAU1^{55(R)}-HA₃ was PCR-amplified using 5'-AAAAGCTAGCGCCACC***ATG***AAACTTGGAAAAA***ACCAATG***-3'

30 (sense, NheI site is underlined, and the Kozak sequence is bold with start codon also italicized) (SEQ ID NO: 174) and, respectively, 5'-

AAA**ACTCGAGCTATTA****CTTGTCGTCATCGTCTTTGTAGT**CCCCCCC

GCACCTCCCACACACAGAC-3' (antisense) (SEQ ID NO: 175), 5'-

AAAACTCGAGCTATTA**CTTGTCGTCATCGTCTTTGTAGT**CCCCCCTGGAATCCC
TGGACTCTGGAAAG-3' (antisense) (SEQ ID NO: 176) or 5'-

AAAACTCGAGCTATTA**CTTGTCGTCATCGTCTTTGTAGT**CCCCC

CGGAGCTGCCCTGGTAAAATCTTTGG-3' (antisense) (SEQ ID NO: 177), where for each
5 antisense primer the XhoI site is underlined and the FLAG sequence introduced by the primer
is bold, underlined and italicized). PCR products were digested using NheI and XhoI and
inserted into pCI-neo (Promega). Each STAU1 plasmid encodes an R323K amino acid change
relative to the NCBI sequence. This change derives from the original pcDNA3/RSV-hSTAU-
HA₃ plasmid.

10 283. To generate pGEX-6p-1-hSTAU1-'RBD'2-RBD3, pRSET B-His-STAU1 was
PCR-amplified using 5'-AAAAGGATCCCCTTTACTTTATCAAGTGG -3' (sense, BamHI
site is underlined) (SEQ ID NO: 178) and 5'-

AAAAGAATTCTTATCACGGTAACTTCTTCAGCTCCTC-3' (antisense, EcoRI site is
underlined) (SEQ ID NO: 179). The PCR product was digested with BamHI and EcoRI and
15 inserted into pGEX-6p-1 (GE Healthcare).

Example 33: Protein Expression in *E. coli* and Protein Purification

284. *E. coli* BL21(DE3) transformed with pGEX-6p-1-hSTAU1-SSM-'RBD'5 was
propagated in multiple 1-liter cultures of Luria Broth supplemented with ampicillin (100 mg/l),
to an O.D.₆₀₀ of ~0.5, at which time 300 µl of 1M isopropyl β-D-1-thiogalactopyranoside was
20 added to each liter and the temperature was reduced from 37°C to 30°C. The following
morning, cells were collected at ~7,000 x g and 4°C and either used directly or flash-frozen in
liquid N₂ for storage at -80°C.

285. Cell pellets were resuspended in ~40 ml of Buffer A (1M NaCl, 25 mM Tris-HCl
pH 8) to which was added 55 µl of 0.93 M dithiothreitol (DTT), 500 µl of 100 mM PMSF, 50 µl
25 of 0.5 M EDTA pH 8, 500 µl of 80 mg/ml lysozyme, and a protease inhibitor tablet
(Roche). Cells were lysed using sonication, and lysates were cleared by centrifugation at 17,000
x g for 30 minutes at 4°C. The soluble portion was removed and loaded on a GStapTMHP
column (GE Healthcare), washed with 1M NaCl, 25 mM Hepes pH 8 (which was sometimes
replaced with Buffer A), washed with gel-filtration (GF) buffer (100 mM NaCl, 10 mM Tris-
30 HCl pH 8, 1.3 mM DTT; this step was sometimes omitted), and then eluted with 0.3 g of
glutathione (reduced, free acid) dissolved in 100 ml of GF buffer. A ~1 mg aliquot of
PreScissionTM Protease (GE Healthcare) was added to ~50 ml of eluted sample and left at 4 °C
overnight. The following day, the sample was applied to a HiTrapTMQ HP column (GE

Healthcare) to remove GST. The flow-through was concentrated to ~ ml using a CORNING® SPIN-X® UF 20 5K column (MW cut-off at 5 kDa), and loaded using an ÄKTA FPLC™ system (GE Healthcare) onto a 120-ml HiLoad™ Superdex™ 75 16/60 prep grade column (GE Healthcare) gel-filtration column that was pre-equilibrated with GF buffer. hSTAU1-SSM-
5 'RBD'5 peak fractions were concentrated as above and used immediately or stored for short periods at 4 °C.

286. Procedures for expressing pGEX-6p-1-hSTAU1-'RBD'2-RBD3 were identical to those for used for expressing hSTAU-SSM-'RBD'5. However, Buffer A contained 5% glycerol and the GSTrap™ column elution was with a solution prepared by dissolving 0.3 g
10 glutathione (reduced, free acid), a protease inhibitor tablet (Roche) and 405 µl of 0.93 M DTT in 100 ml of GF buffer. After PreScission™ Protease treatment overnight, the solution was loaded onto a HiTrap™ SP FF column (GE Healthcare) and eluted using a linear NaCl gradient of GF buffer and glycerol-containing Buffer A using a BioLogic DuoFlow™ FPLC system. Peak fractions were collected, concentrated as above, and loaded onto a HiTrap™ Q HP
15 column to remove contaminating RNAs. The flow-through was concentrated and loaded on a 120 ml HiLoad™ Superdex™ 200 16/60 prep grade column (GE Healthcare) that was equilibrated with GF buffer containing 2.97 mM DTT using the BioLogic DuoFlow™ FPLC system.

Example 34: Analytical Ultracentrifugation

20 287. hSTAU1-SSM-'RBD'5 was purified as above, except the final GF buffer contained 2.97 mM DTT, and submitted to the University of Connecticut Analytical Ultracentrifugation Facility for sedimentation velocity analysis. A Beckman-Coulter XL-I analytical ultracentrifuge with double-sector synthetic boundary cells having sapphire windows was used to take interference scans. Measuring refractive index rather than absorbance was
25 especially useful considering the low extinction coefficient at A₂₈₀ that typifies SSM-'RBD'5; which lacks tryptophan residues. Interference scans were collected at 55,000 RPM and 20 °C every minute for 7 hours. Data were analyzed using: 1) DcDt+, version 2.0.9 (Philo, 2000; 2006), to determine the sedimentation coefficient distribution that was independent of a model, 2) Sedfit, version 10.09beta (Schuck, 2000), to produce a model-based continuous
30 sedimentation coefficient distribution using the Lamm equation or c(s) to identify the number of species (e.g., monomers vs. dimers) in solution, and 3) Sedanal, version 5.60 to combine datasets from the three highest of four concentrations tested, perform a global analysis, and determine the protein association model using the Lamm equation.

Example 36: Size Determination Using Gel-Filtration Chromatography

288. Size standards were prepared by dissolving dried proteins in 2 ml of GF buffer containing 2.97 mM DTT. Proteins consisted of 3.8 mg of conalbumin (75 kDa), 2.3 mg carbonic anhydrase (29 kDa) and 6.7 mg aprotinin(6.5kDa), each from the Low Molecular Weight Gel Filtration Calibration Kit (GE Healthcare; #28-4038-41), and 6mg of lysozyme (14.3kDa) (Sigma; #L6876-10G). The dissolved solution (1 ml, determined using a 1ml loop) was loaded onto a 120 ml HiLoad™ Superdex™ 200 16/60 prep-grade column (GE Healthcare) and separated at a 1 ml/min flow rate using the BioLogicDuoFlow™ FPLC system. For size estimations, gel-filtration of SSM-‘RBD’5 (WT), SSM-‘RBD’5 (E424H, D427V) and ‘RBD’2-RBD3 was performed as described for the size standards. SSM-‘RBD’5 (WT) was loaded at 8 mg/ml, SSM-‘RBD’5 (E424H, D427V) was loaded, and ‘RBD’2-RBD3 was loaded at 6 mg/ml.

Example 37: Extended Protein Crystallization and Structure Determination**Procedures**

289. Gel-filtration -purified hSTAU1 SSM-‘RBD’5 was screened for crystal formation using the JCSG Core Suites I-IV (QIAGEN; Lesley and Wilson, 2005) and a Mosquito robot. Using the sitting drop method and CrystalQuick™ (Greiner) plates, 0.2 µl of protein in GF buffer (10 mM Tris-HCl pH 8, 100 mM NaCl, and 1.3 mM DTT) at 15 mg/ml (determined using the Bradford assay) was mixed with 0.2 µl of reservoir solution, which was 70 µl. Plates were covered with ClearSealFilm™ and incubated at 18 °C. After four days, bi-pyrimidal shaped crystals (Figure S11) were found using JCSG Core Suite II condition #96 (or H12). The screening solution was dehydrated ~1.3 fold and therefore contained 130 mM citric acid pH 2.5 and 26% (w/v) PEG 6000 with a final pH of 4.

290. Using the hanging drop method and the Stanford Synchrotron Radiation Lightsource (SSRL), a native crystal dataset was generated using the conditions described above, except that drops were 3 µl and the reservoir contained 1 ml of 0.1 M citric acid pH 2.5 and 35% PEG 6000. Crystals (20-30 µl), the largest of which were on average 240 µm tip-to-tip, were transferred to a 50/50 (v/v) mixture of silicon and PARATONE® N oils, moved around in the cryoprotectant with a nylon loop until free of phase-separated sweat, collected with the loop, and flash-frozen in liquid N₂. The native crystal dataset was collected under a cryostream remotely at the SSRL beamline 9-2 on a MAR-325 CCD detector (Table 12).

291. A second native dataset was collected at the Cornell High Energy Synchrotron Source (CHESS) on a crystal produced similarly to the SSRL dataset crystal, except 3-1 drops were made by mixing 1.5 µl of hSTAU1 SSM-‘RBD’5 protein at a concentration of 5 mg/ml

(determined at A_{280} with an ϵ_{280} of 4,470) with 1.5 μ l of crystallization buffer (127 mM citric acid pH 2.5 and 25.9% PEG 6000 (final pH 4)). Drops were placed on a Micro-Bridge (Hampton Research, HR3-310) over 1 ml of 118 mM citric acid pH 2.5 and 29% PEG 6000 in the sealed wells of a 24-well plate. Data were collected under a cryostream at the CHESS beamline F1 on an ADSC Q-270 CCD detector (Table 12).

292. The crystal used for SAD phasing was produced similarly to the CHESS native crystal, but using a 4 μ l-drop and a reservoir of 130 mM citric acid pH 2.5 and 26.5% PEG 6000. A 10 μ l soaking-solution of 10 mM ethyl mercuric phosphate (EMP; Hampton Research, HR2-446) in 127 mM citric acid pH 2.5 and 28.2% PEG 6000 (final pH 4) was added directly to the drop containing the crystal, and a thick glass coverslip was placed over the Micro-Bridge. After ~45 min at room temperature, the crystal was transferred to 20 μ l of cryoprotectant solution and flash-frozen. Data were collected in-house under a cryostream using a Bruker AXS X8 Prospector Ultra X-ray generator system equipped with an APEX II CCD detector (Table 12).

293. Data integration and scaling were performed using HKL2000 for both native synchrotron datasets. Alternatively, PROTEUM2 (Bruker AXS Inc.) was used for the in-house SAD dataset (Table 12).

294. CTRUNCATE within CCP4 (CCP4, 1994) was used to convert the SAD dataset intensities for input into Autosol within Phenix, where anomalous signals identified two Hg sites at each of the only two Cys residues. The resulting density-modified Resolve map and the FFFEAR and FFJOIN programs were used to locate α -helices. Combinations of Autobuild, hand-building with Coot, and rounds of refinement using Phenix at this and all subsequent stages yielded a low-resolution model of 'RBD'5 and the SSM two α -helices. A molecular-replacement (MR) search using this model, Phaser (McCoy et al., 2007), and the 1.7 Å SSRL dataset was unsuccessful. However, a solution was found using the 2.2 Å CHESS dataset (Table 12). Following further refinement assisted by placing a homology model for 'RBD'5 using I-TASSER (Roy et al., 2010), a MR solution was obtained for the 1.7 Å SSRL dataset and used the final stages of refinement. Structure figures were generated using PyMOL (Schrödinger, LLC).

Exmple 38: Western Blotting, RT-PCR and Immunoprecipitations

295. Protein was electrophoresed in SDS-polyacrylamide, transferred to Hybond ECL nitrocellulose (Amersham), and probed with antibodies that recognize FLAG (Sigma), HA (Roche), calnexin (StressGen), UPF1 (Gong et al. 2009), STAU1, RFP (Abcam), GFP

(Abcam) or STAU2 (Sigma). Immunoreactivity was assessed using SuperSignal West Pico or Femto (Pierce Biotechnology). After autoradiography, films were quantitated using ImageQuant (Molecular Dynamics).

296. Reverse transcriptions (RTs) and PCR amplifications were performed as
5 previously described. RT-PCR products were electrophoresed in 5% polyacrylamide and quantitated by PhosphorImaging (Molecular Dynamics).

297. Immunoprecipitations were performed using anti-GFP (Abcam), anti-HA (Roche) or anti-FLAG (Sigma).

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SEQ ID NO: 1 Stau1 (Stau1) (Genbank No: BC050432) nucleotide sequence

- 1 aaccacttaa ccttcagaa ctgaacaaag acaacattgt tctggaacg ccctctttt
 61 aaaaaaaggt agaacttag acttcatagc actgaattaa cctgcaactga aagctgttta
 121 cctgcatttg ttcacttttg ttgaaagtga ccatgtctca agttcaagtg caagttcaga
 30 181 acccatctgc tgctctctca gggagccaaa tactgaacaa gaaccagtct cttctctcac
 241 agcctttgat gattattcct tctactacta gctctctgcc ctctgaaaat gcaggttagac
 301 ccattcaaaa ctctgcttta cctctgcat ctattacatc caccagtgcg gctgcagaaa
 361 gcataacccc tactgtagaa ctaaatgcac tgtgcatgaa acttggaata aaaccaatgt
 421 ataagcctgt tgacccttac tctcggtatg agtcaccta taactacaac atgagaggag
 35 481 gtgcttatcc cccgaggtac tttaacccat ttccagttcc accttactt tatcaagtgg
 541 aactttctgt gggaggacag caatttaatg gcaaaggaaa gacaagacag gctgcgaaac
 601 acgatgctgc tgccaaagcg ttgaggatcc tgcagaatga gccctgcca gagaggctgg
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 40 781 caccaccat gaagaacttt gtgaccaagg ttctggttgg ggagtttgg ggggaagggtg
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 45 1081 agcgaggcct cccgcgccgc agggagtttg tgatgcaggt gaaggttga aaccacactg

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 5 1381 atcagcagct gcctgctgga attcttccca tgggtcccga ggtcgcccag gctgtaggag
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 1501 cggtaactgc catgatagcc cgagagtgtg tgtatggggg cacctcgccc acagccgaga
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 15 1981 gggccgagag acacgggtggg taaaggatgt gggcagcagc aggggaagaca acagaaacac
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SEQ ID NO: 2 Staufen1 (Stau1) (Genbank No: BC050432) amino acid sequence

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 YQVELSVGGQQFNGKGKTRQA AKHDA AAKALRILQNEPLPERLEVNGRESEENLNKSEISQ
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 REFVMQVKVGNHTAEGTGTNKKVAKRNA AENMLEILGFKVPQAQPTKPALKSEEKTPIKKP
 45 GDGRKVTFFEPGSGDENGT SNKEDEF RMPYLSHQQLPAGILPMVPEVAQAVGV SQGHHTKD
 FTRAAPNPAKATVTAMIARELLYGGTSPTAETILKN NISSGHVPHGPLTRPSEQLDYLSRVQGF
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50 SEQ ID NO: 3 ALU element consensus sequence

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 55 TCGCGCCACTGCACTCCAGCCTGGGCGACAGAGCGAGACTCCGTCTCAAAAAAAAAA

V. CLAIMS

What is claimed is:

1. A method of identifying a Stau1 (Stau1)-binding site (SBS) that mediates Stau1-mediated mRNA decay (SMD) comprising the steps of
 - 5 a. Down-regulating Stau1 or an ALU element-containing long noncoding RNA (lncRNA);
 - b. Identifying transcripts that are up-regulated at least 1.8-fold upon downregulation of Stau1 or the ALU element-containing lncRNA, wherein transcripts that are up-regulated at least 1.8-fold are SMD targets;
 - 10 c. Identifying an ALU element within the 3' UTR of the transcript of the SMD target, wherein the ALU element comprises a first strand of the SBS.
2. The method of claim 1, wherein the Stau1 or ALU element-containing lncRNA is down regulated by siRNA.
3. The method of claim 1, wherein the SMD target is selected from the group consisting of
 - 15 IFI44, FBLIM1, CLDN11, PDLIM3, OASL, TAF7L, NOX4, AIM1, SERPINE1, DCP2, EIF5A2, CEP135, LRRFIP1, ZFP90, TP53, PHLPP2, EHHADH, CCDC125, NUA2, AKT2, APPL1, COL16A1, RIPK1, MGC14799, GNAS, DFFA, CSDA, SEC61A1, PSMD12, , C4orf9, FLJ10613, GNE, FLJ30656, LOC149603, TEGT, MCM4, LOC63929, KIAA0186, PRKAR2A, NUTF2, GDF1, PAICS, TMPO, AAMP, IF, FN1, IFIT2, SCG2,
 - 20 STMN3, GAP43, CKMT1, OSF-2, FLJ25348, GNG4, FLJ33505, FBLP-1, IGFBP5, ITGB3, IFIT4, ALP, TAGLN, TFPI2, CTNNAL1, THBS1, PTPRO, CEB1, MGC29643, FLJ20035, AIM1, C14orf141, DUSP6, G1P2, FLJ20637, SLAMF1, SCLY, IL7R, ARHGDIB, ACTA2, CXCL1, RIG-1, IFIT1, FLY34064, MGC19764, TLE4, DACT1, C9orf39, JUN, IGA4, TNC, FLJ13621, MICAL2, TMSNB, PTGER4, C14orf128, GDAP1,
 - 25 ZFP36L1, UNG, CDKAL1, ALDH1A3, PSMB9, PHF11, BANK1, KIAA0143, GBP1, EPPK1, ACTN1, RNF20, LRRFIP1, LOC150759, DPYSL3, PSK-1, ZFP90, TP53, LOC148418, TGFB1I1, ETV1, ARHGAP19, SOCS2, CDK5R1, SDS3, SCDGF-B, HDAC8, HMGB3, KIAA0931, DFKZp761K1423, PTPRF, TXNRD3, CGI-72, MGC15634, HES1, C11orf9, and TOIA1.
- 30 4. The method of claim 1, wherein the up-regulation of the SMD target is determined by microarray.

5. The method of claim 1, wherein the up-regulation of the SMD target is determined by western blot.

6. The method of claim 1, wherein when Stau1 is down-regulated, the method further comprises step d. identifying an ALU element in a cytoplasmic polyadenated lncRNA that contains an ALU element that base pairs with the ALU element in the SMD target, wherein the ALU element of the lncRNA comprises a second strand of the SBS.

7. The method of claim 6, wherein the lncRNA is identified by co-immunoprecipitation.

8. The method of claim 6, wherein the lncRNA is identified by computational analysis.

9. A method of identifying a SBS of an SMD target comprising

- 10 a. transiently expressing an ALU element-containing lncRNA linked to a reporter gene;
- b. immunoprecipitating the ALU element-containing lncRNA using an anti-reporter protein antibody;

wherein ALU elements within RNA that encode proteins other than Stau1 that are co-immunoprecipitated with the ALU element of the lncRNA is a SBS of an SMD target.

10. A method of screening for an agent that modulates SMD comprising

- a. incubating the agent with a stably transfected cell comprising a reporter gene with a 3' UTR ALU element; a lncRNA containing a corresponding ALU element; and Stau1, and
- 20 b. assaying the amount of mRNA of the reporter gene in the cell, wherein a increase or decrease in the amount of mRNA of the reporter gene relative to the amount of mRNA of the reporter gene in the absence of the agent indicates a substance that modulates SMD activity.

11. The method of claim 10, wherein the Stau1 has at least 80%, 85%, 90%, or 95% identity to the sequence set forth in SEQ ID NO: 1, or a fragment thereof.

12. The method of claim 10, wherein the SMD target comprises at least one ALU element in its 3' UTR.

13. The method of claim 12, wherein the ALU element has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% identity to the consensus ALU element set forth in SEQ ID NO: 3.

14. A method of screening for an agent that modulates SMD comprising

- a. incubating the agent with an SMD target and Stau1 forming a substance-SMD target-Stau1 mixture, and
- b. assaying the amount of SMD target-Stau1 complex present in the mixture,
5 wherein a increase or decrease in the amount of SMD target -Stau1 complex relative to the amount of SMD target -Stau1 complex in the absence of the agent indicates that the substance promotes or inhibits SMD, respectively.

15. The method of claim 14, wherein the Stau1 has at least 80%, 85%, 90%, or 95% identity to the sequence set forth in SEQ ID NO: 1, or a fragment thereof.

- 10 16. The method of claim 14, wherein the SMD target comprises at least one ALU element in its 3' UTR.

17. The method of claim 16, wherein the ALU element has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% identity to the consensus ALU element set forth in SEQ ID NO: 3.

- 15 18. A method of screening for an agent that modulates SMD comprising,

- a. incubating the agent with an SMD target and Stau1 forming a substance-SMD target-Stau1 mixture, and
- b. assaying the amount of SMD target present in the mixture, wherein a
20 increase or decrease in the amount of SMD target relative to the amount of SMD target in the absence of the agent indicates that the substance inhibits or promotes SMD, respectively.

19. The method of claim 18, wherein the Stau1 has at least 80%, 85%, 90%, or 95% identity to the sequence set forth in SEQ ID NO: 1, or a fragment thereof.

20. The method of claim 18, wherein the SMD target comprises at least one ALU element
25 in its 3' UTR.

21. The method of claim 20, wherein the ALU element has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% identity to the consensus ALU element set forth in SEQ ID NO: 3.

22. An agent that modulates SMD, wherein the substance is an siRNA that modulates SMD.

- 30 23. The agent of claim 22, wherein the siRNA binds Stau1.

24. The agent of claim 22, wherein the siRNA binds to an ALU element.
25. The agent of claim 24, wherein the ALU element is located within a cytoplasmic and polyadenylated long noncoding RNA.
26. The agent of claim 24, wherein the ALU element is located within the 3' UTR of an SMD target.
27. A method of identifying an agent that binds an SBS comprising contacting the SBS with the agent to be screened, wherein the SBS comprises an ALU element from a SMD target and an ALU element from a lncRNA that base pairs with the ALU element from the SMD target.
28. The method of claim 27, wherein the ALU element from the SMD target has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% identity to the consensus ALU element set forth in SEQ ID NO: 3.
29. A method of identifying genes modulated by the down-regulation of an ALU element-containing lncRNA comprising
- a. incubating a agent that down-regulates SMD with a stably transfected cell comprising Stau1 and one or more selected genes comprising one or more nonsense-mutations, and
 - b. assaying the amount of protein expressed of the gene being screened or mRNA present for the gene being screened, wherein a increase or decrease in the amount of protein or mRNA relative to the amount of protein or mRNA in the absence of the siRNA indicates a gene that is modulated by Stau1 activity.
30. The method of claim 29, wherein the agent that down regulates SMD is siRNA directed against the ALU element-containing lncRNA.
31. The method of claim 29, wherein the gene is up-regulated by down-regulating the lncRNA.
32. The method of claim 29, wherein the amount of mRNA present is measured by RT-PCR.
33. The method of claim 29, wherein the amount of protein expression is measured by western blotting.

34. A method of treating a disorder associated with expression of a truncated or erroneous protein in a subject comprising administering to the subject an agent, wherein the agent increases SMD of the gene encoding the target protein in the subject.

35. The method of claim 34, wherein the SMD occurs in the pioneering round of translation.

5 36. The method of claim 34, wherein the SMD targets eIF4E-bound mRNA during steady-state mRNA translation.

37. The method of claim 34, wherein the disorder is an inherited genetic disorder.

38. The method of claim 37, wherein the genetic disorder can be selected from the group consisting of cystic fibrosis, hemophilia, mucopolysaccharidoses, muscular dystrophy,
10 anemia, glycolytic enzyme deficiency, connective tissue disorder, DNA repair disorder, dementia, Diabetes mellitus type II, Alzheimer's disease, Marfan's syndrome, β -Thalasimia, Neurofibromatosis, Hypercholesterolemia, Sandhoff disease, epidermolysis bullosa simplex, insulin resistance, maple syrup urine disease, hereditary fructose intolerance, inherited immunodeficiency, inherited cancer, carbohydrate metabolism
15 disorder, amino acid metabolism disorder, lipoprotein metabolism disorder, lipid metabolism disorder, lysosomal enzymes disorder, steroid metabolism disorder, purine metabolism disorder, pyrimidine metabolism disorder, metal metabolism disorder, porphyrin metabolism disorder, and heme metabolism disorder.

39. The method of claim 34, wherein the disorder is an acquired disorder.

20 40. The method of claim 34, wherein the disorder is dementia.

41. The method of claim 34, wherein the acquired disorder is a cancer.

42. The method of claim 41, wherein the cancer is selected from the group consisting of lymphoma, B cell lymphoma, T cell lymphoma, leukemia, carcinoma, sarcoma, glioma, blastoma, neuroblastoma, Ewing's sarcoma, gastric cancer, lung cancer, plasmacytoma,
25 histiocytoma, melanoma, mycosis fungoide, hypoxic tumor, myeloma, metastatic cancer, bladder cancer, brain cancer, nervous system cancer, head and neck cancer, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, liver cancer, colon cancer, cervical cancer, breast cancer, epithelial cancer, renal cancer, genitourinary cancer, pulmonary cancer, esophageal carcinoma, hematopoietic cancers, testicular cancer, and colorectal cancer.

30 43. The method of claim 34, wherein the mRNA transcript of the truncated or erroneous protein comprises a single ALU element in the 3' UTR.

44. The method of claim 34, wherein the agent is Stau1 or a complex comprising Stau1 and Upf1.

45. The method of claim 34, wherein the agent is an ALU element-containing lncRNA that base pairs with the ALU element in the mRNA of the 3' UTR of the truncated or erroneous
5 protein.

46. The method of claim 34, wherein the agent is a functional nucleic acid, siRNA, peptide, protein, antibody, or small molecule.

10

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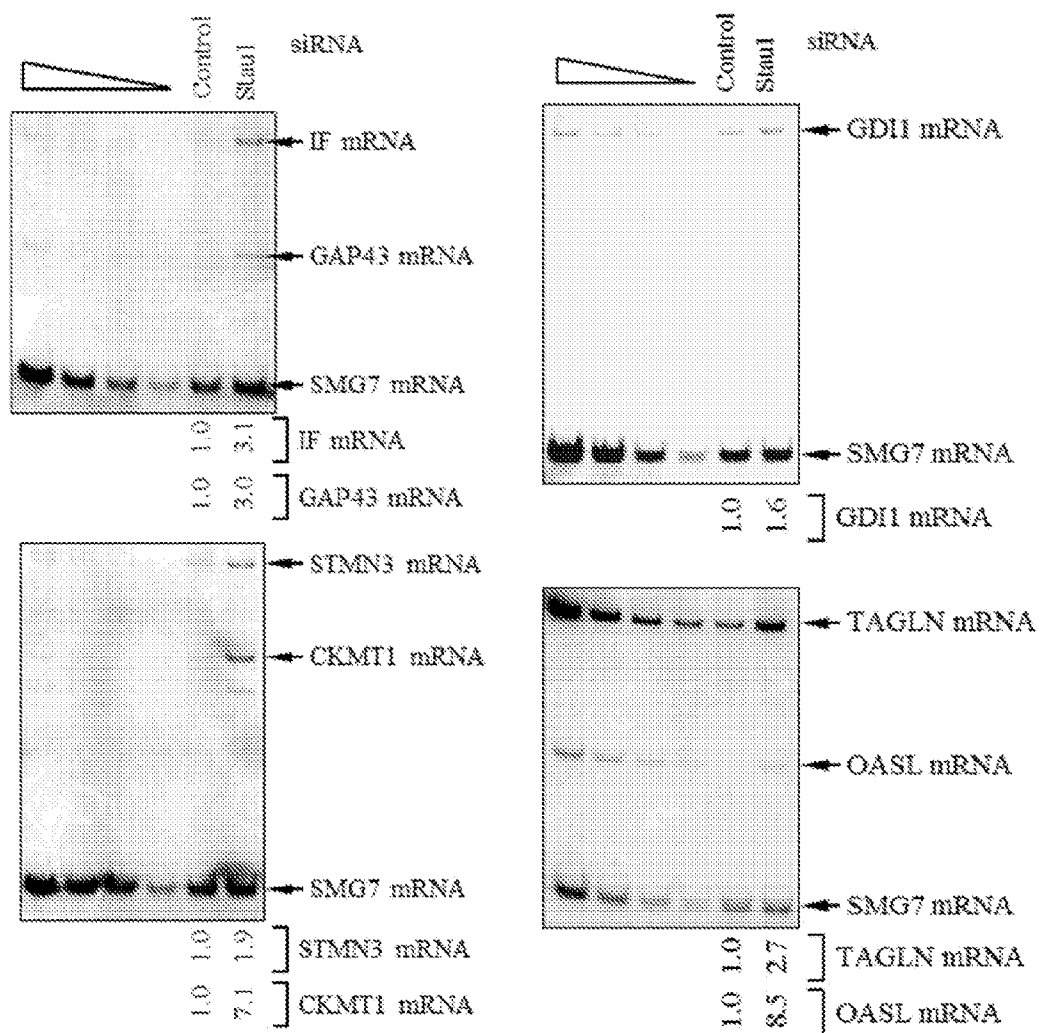
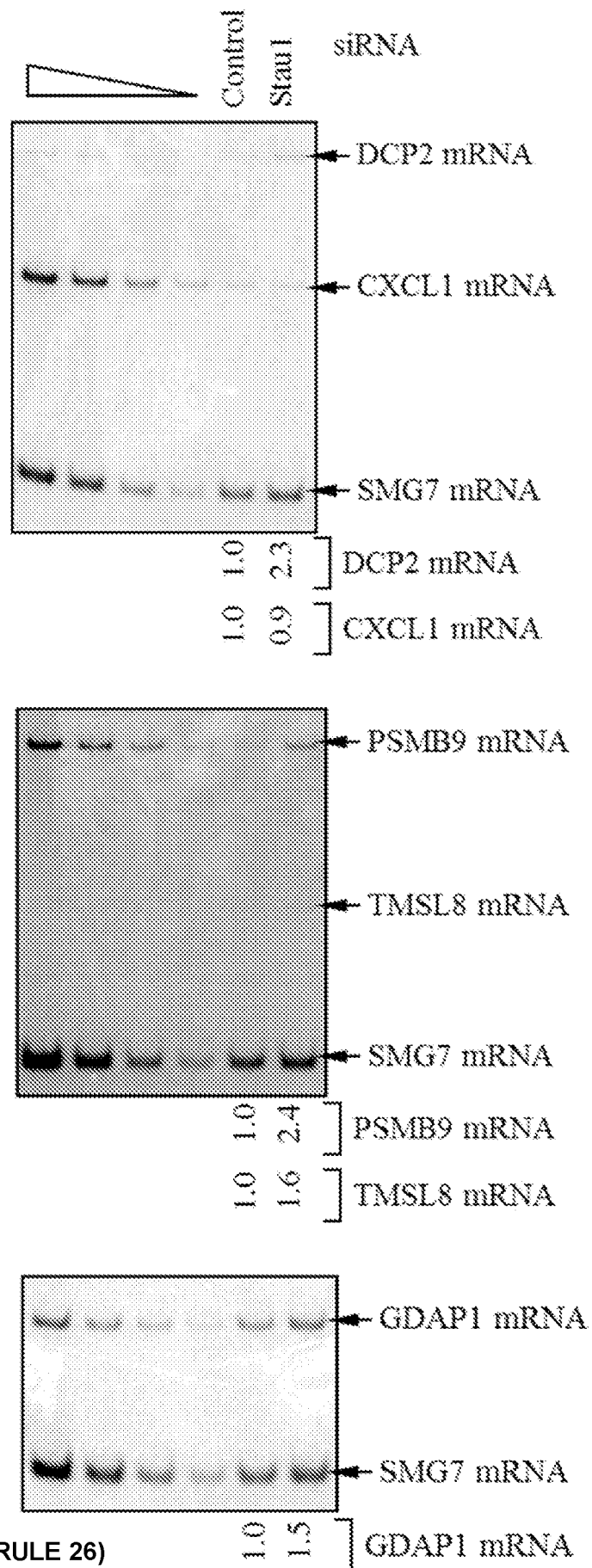


FIG. 1-1

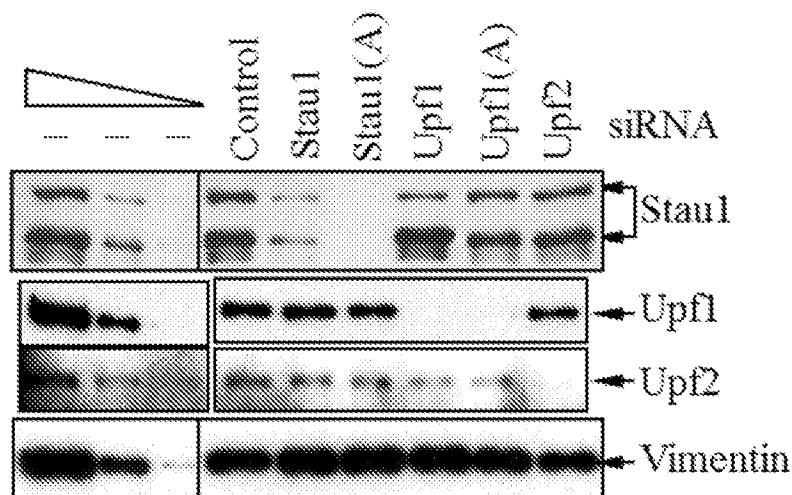
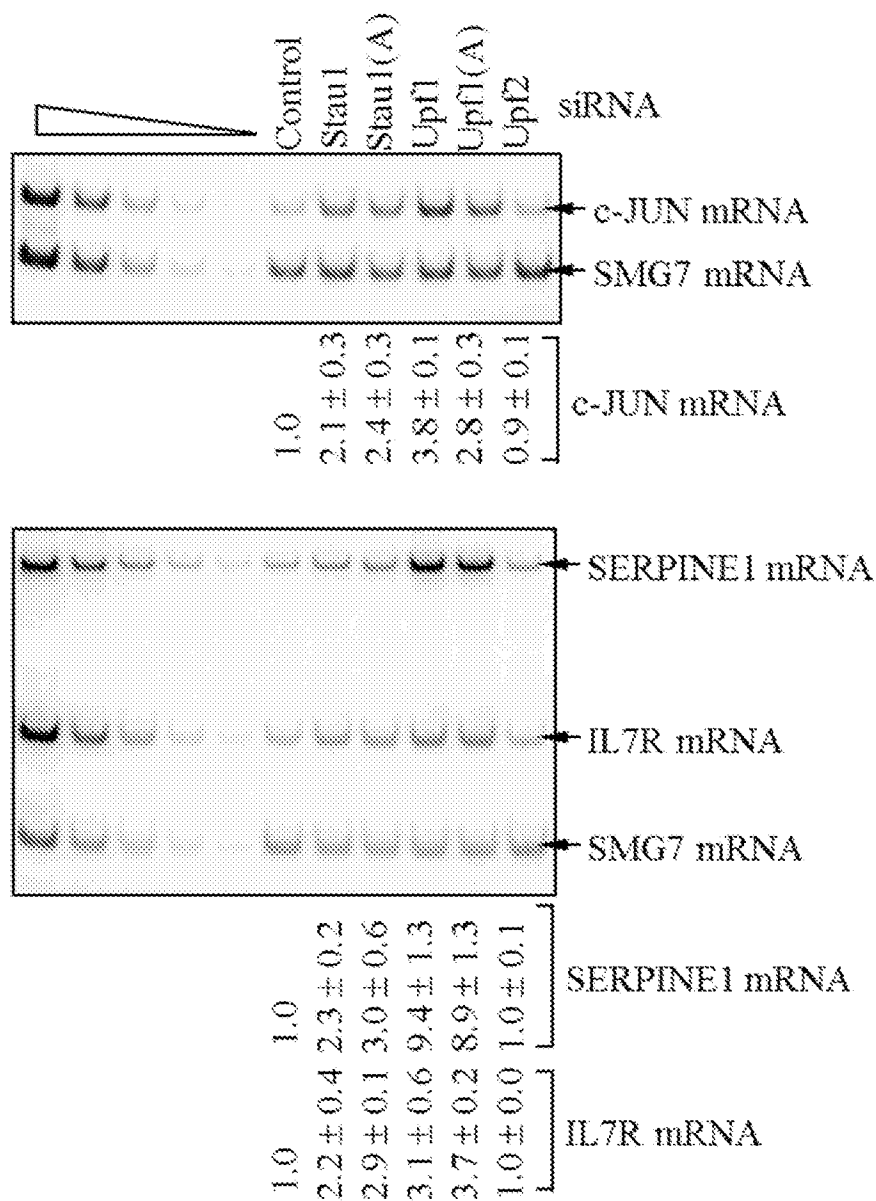
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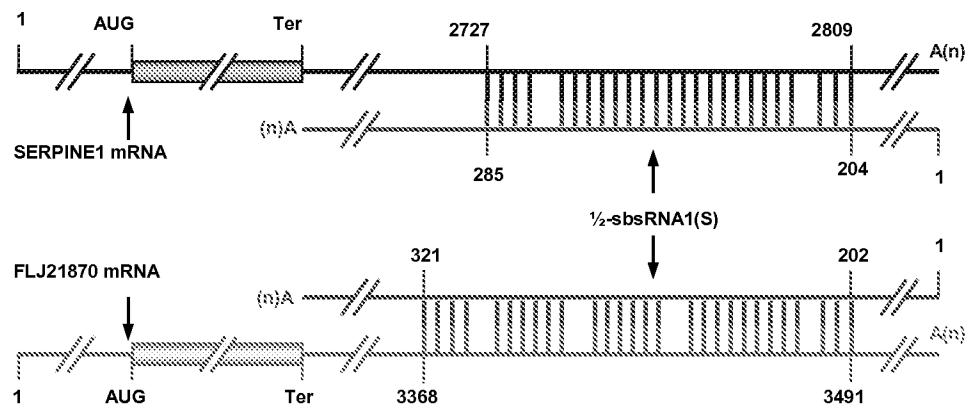
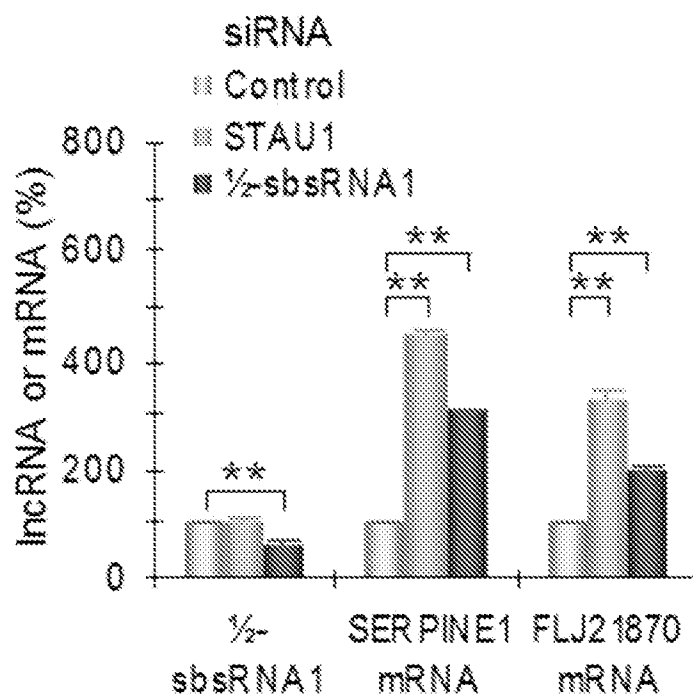
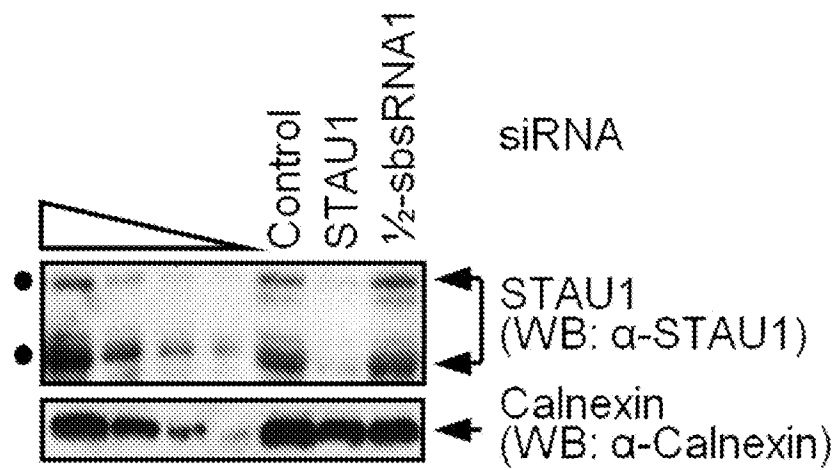
FIG. 1-2

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A**B****FIG. 2**

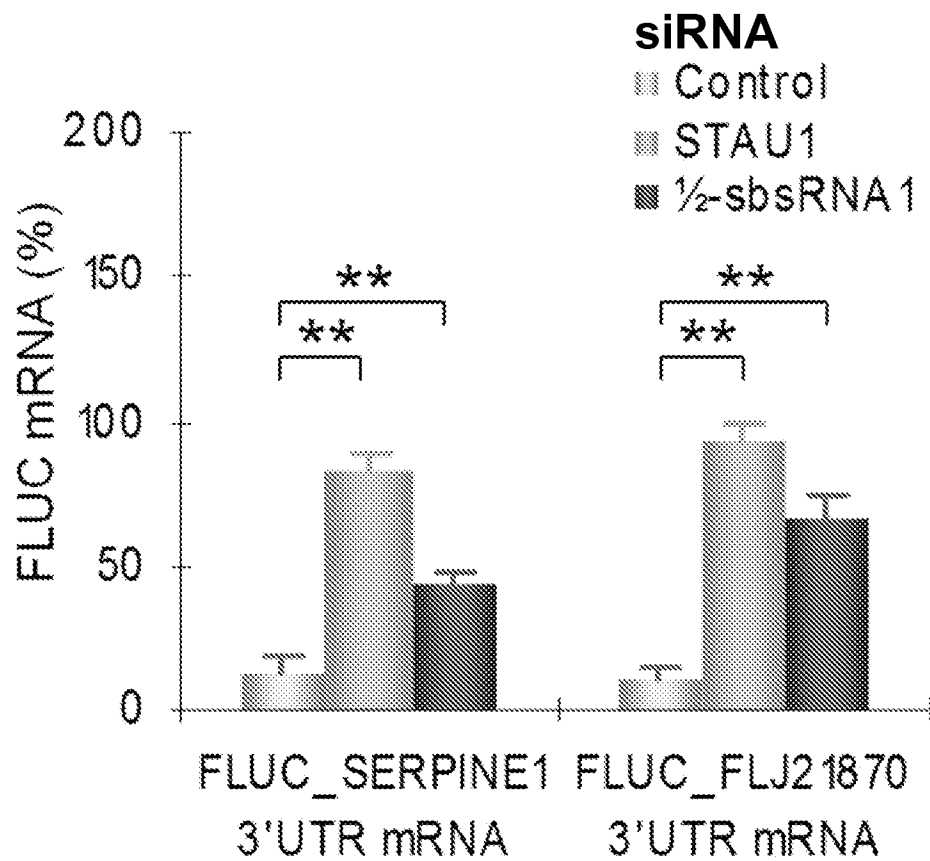
SUBSTITUTE SHEET (RULE 26)

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A**B****FIG. 3**

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C



D

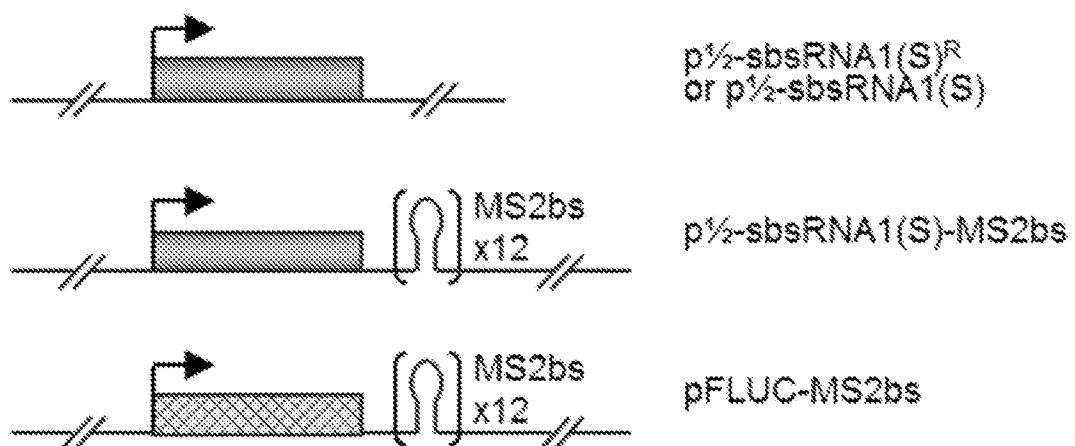


FIG. 3

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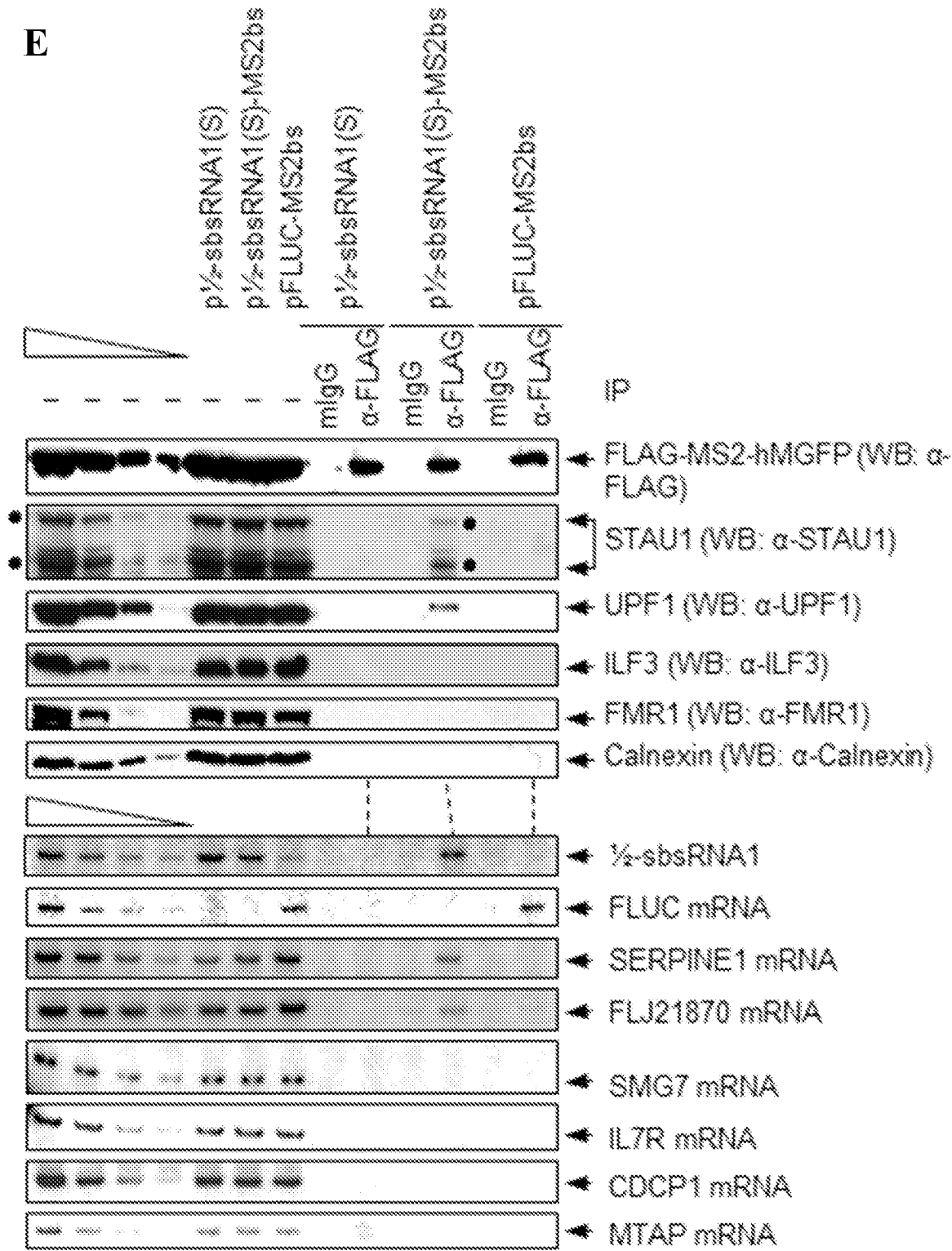


FIG. 3

SUBSTITUTE SHEET (RULE 26)

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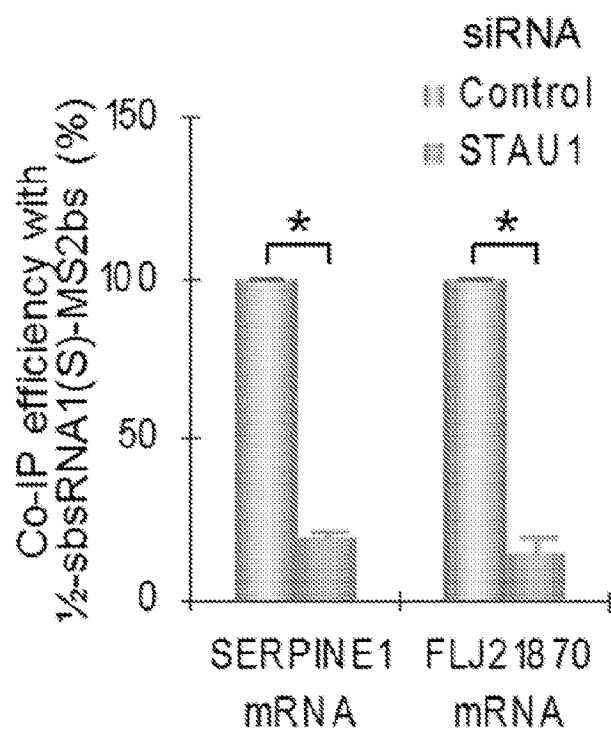
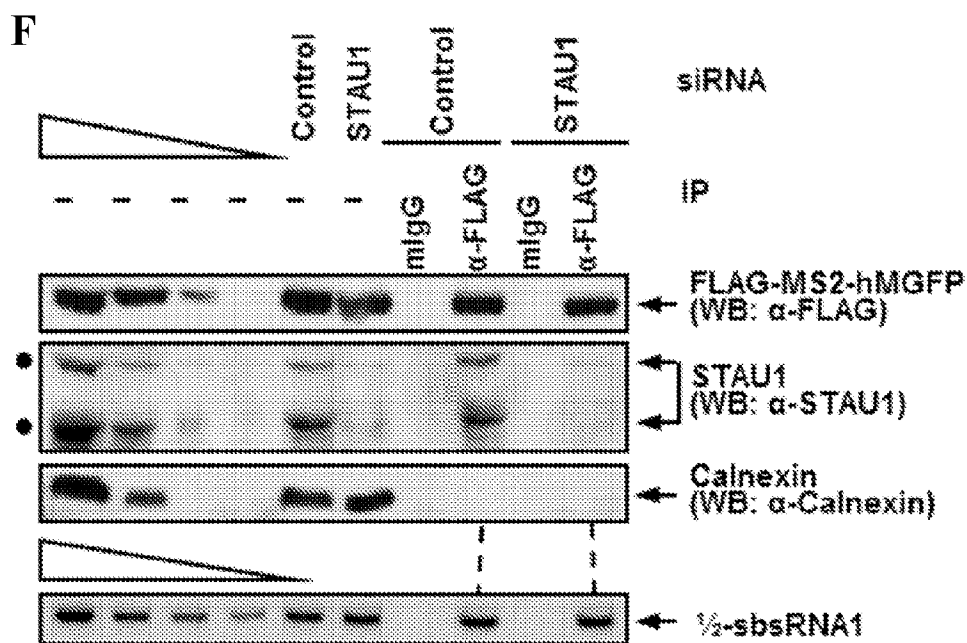


FIG. 3

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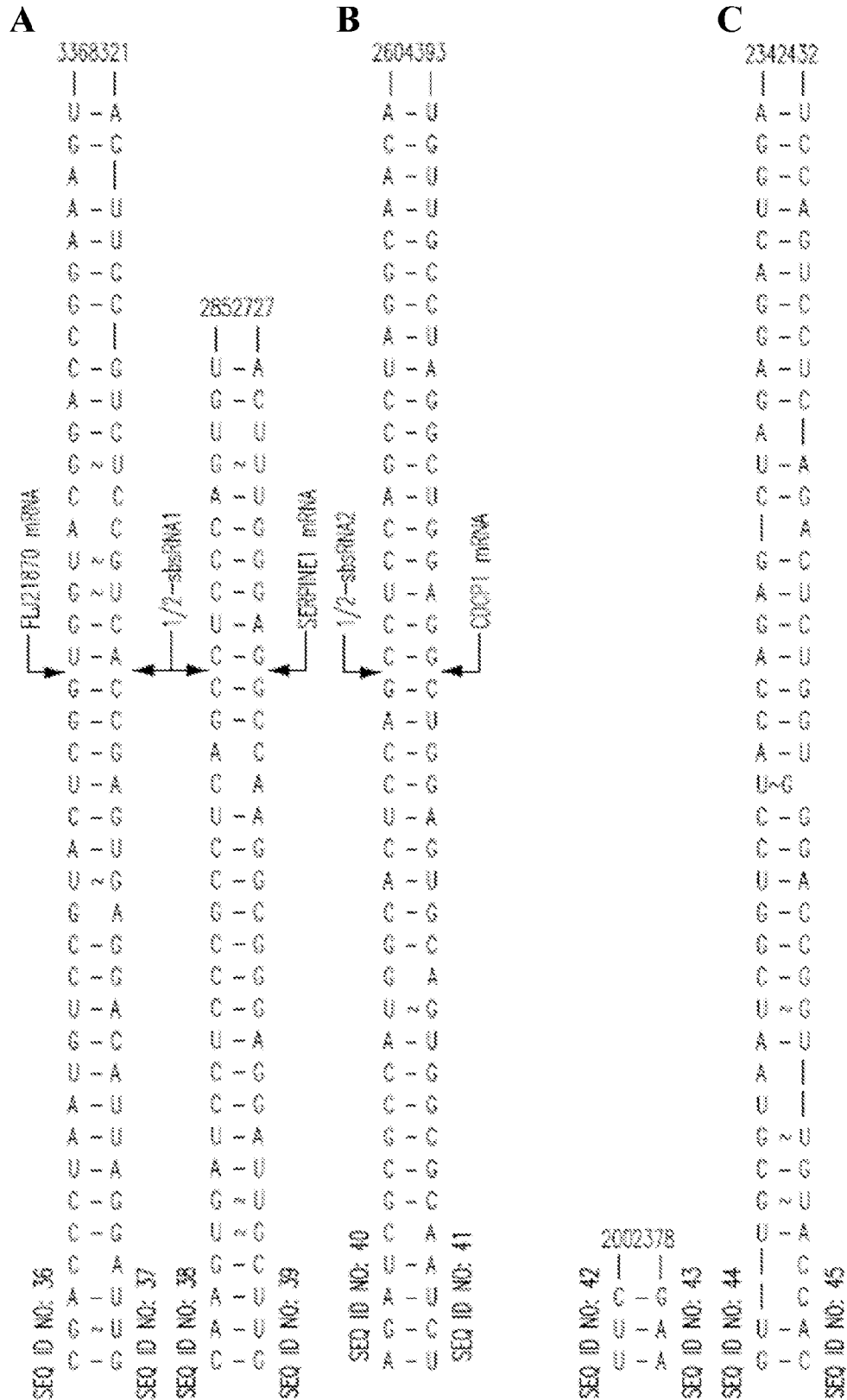


FIG. 4-1

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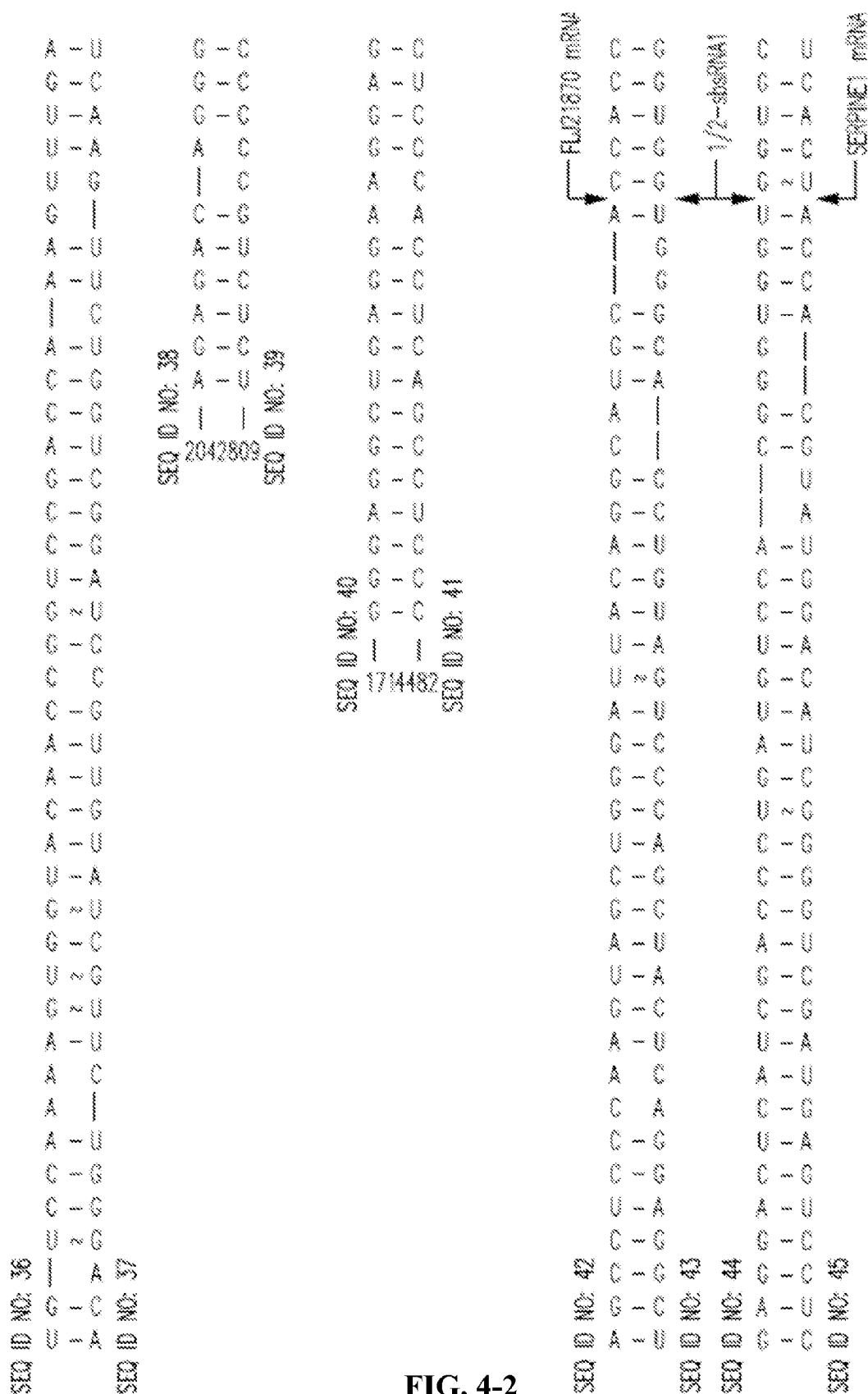


FIG. 4-2

10 / 39

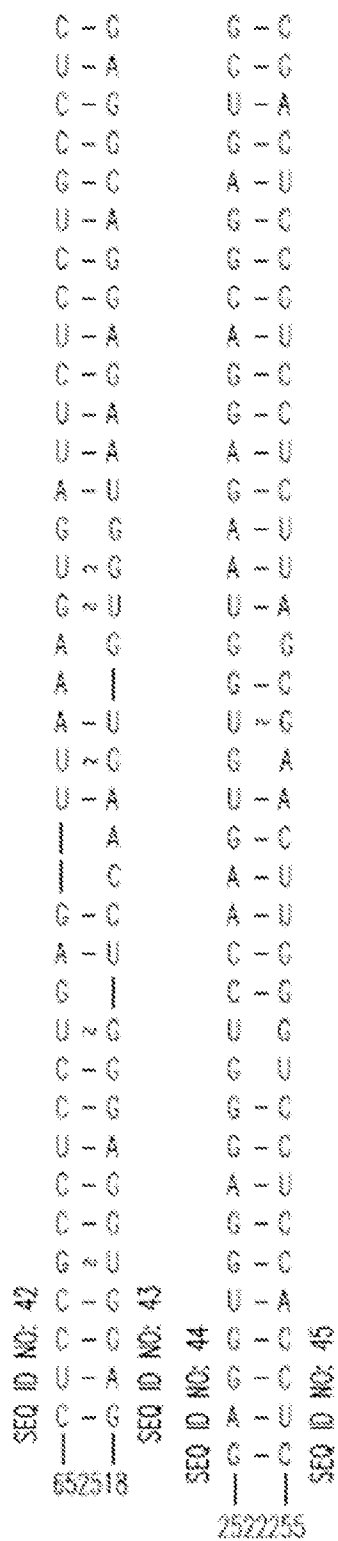
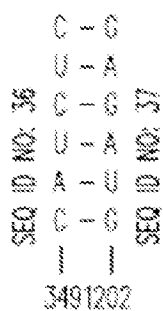


FIG. 4-3

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D

3368504

G	C
G	C
C	C
C	A
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C	C
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A	U
U	A
G	C
C	C
U	A
G	C
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A	U
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C	C
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A	U
G	C
C	C
A	U

SEQ ID NO: 48 SEQ ID NO: 49

FIG. 4-4

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	G - C
	G - C
	G - C
	A - U
	G - C
	G - C
	C - G
	C - G
	G - C
	A - U
	G - C
	G - C
	C - G
	A - U
	G - C
	G - C
	U - G
	G - C
	G - C
	A - U
	U - A
	C - G
	A - U
	C - G
	U ~ G
	U - A
	G - C
	A - U
	G - C
	G - C
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SEQ ID NO: 46

2303446

I I

C - C

C - C

U - A

C - C

A - U

A - U

C - U

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U - A

U ~ G

U - A

SEQ ID NO: 49

FIG. 4-5

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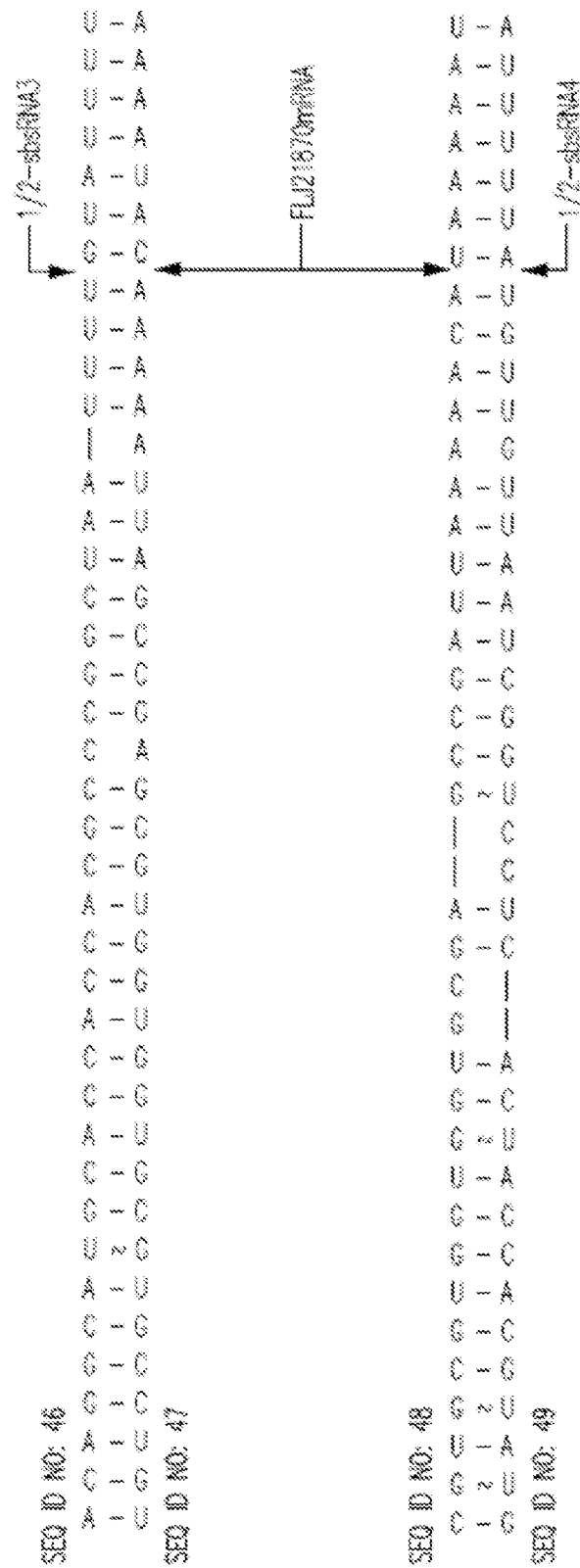


FIG. 4-6

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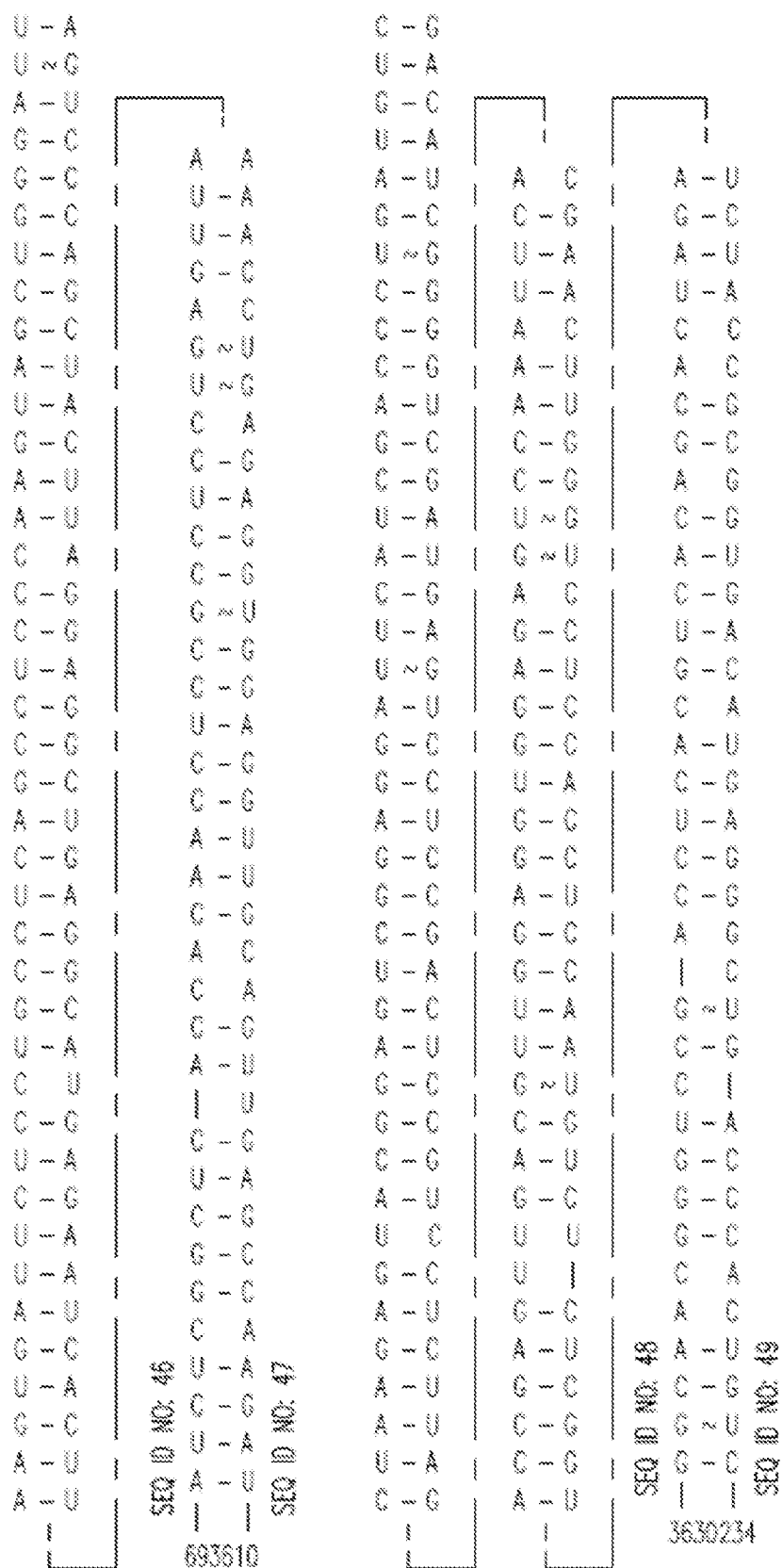


FIG. 4-7

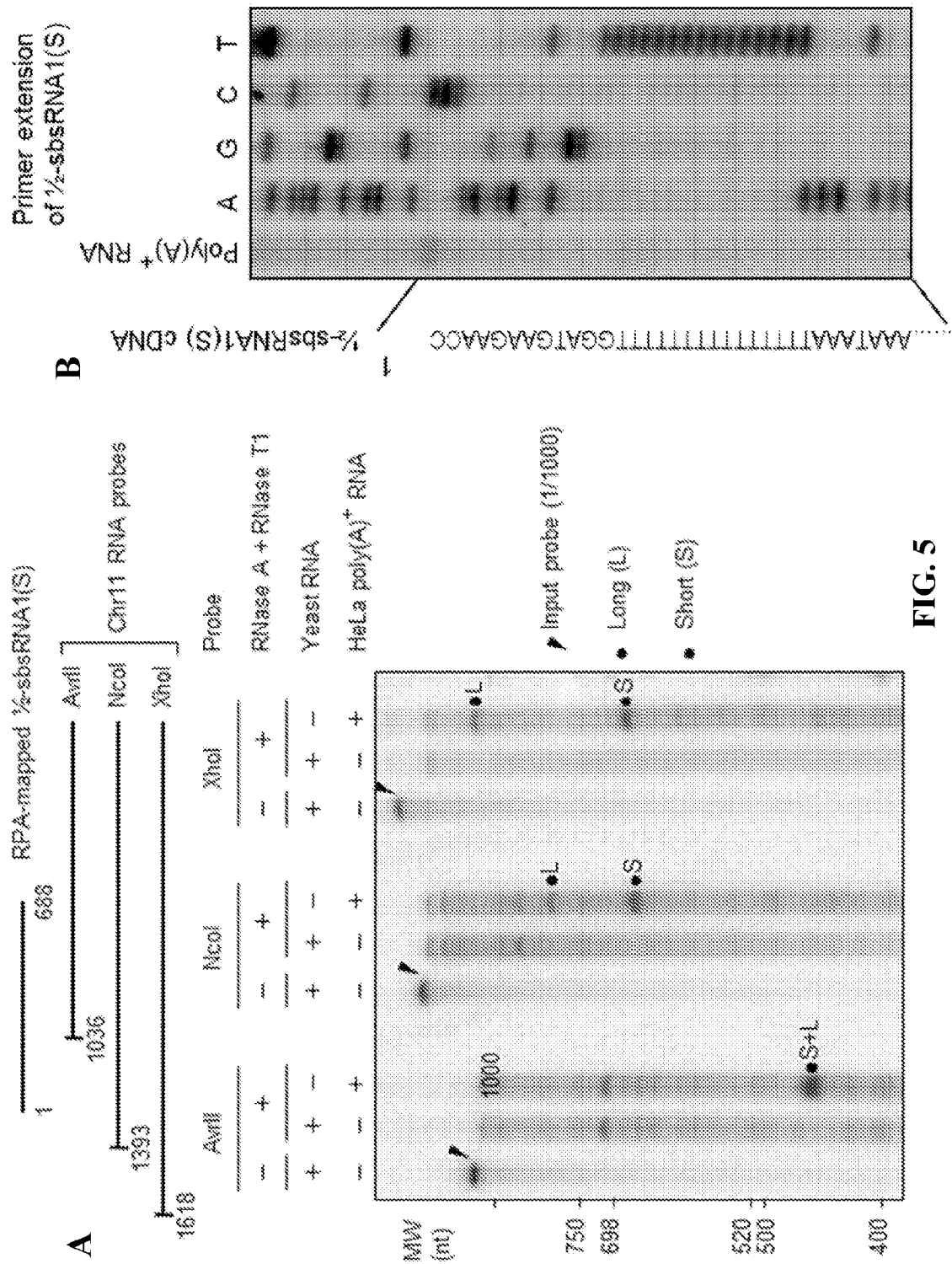


FIG. 5

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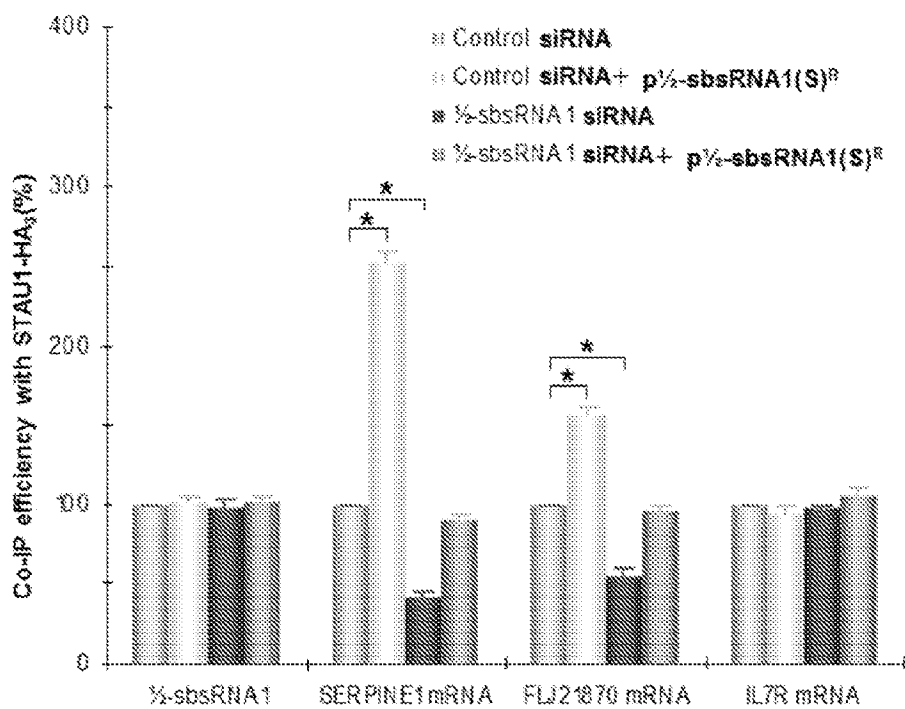
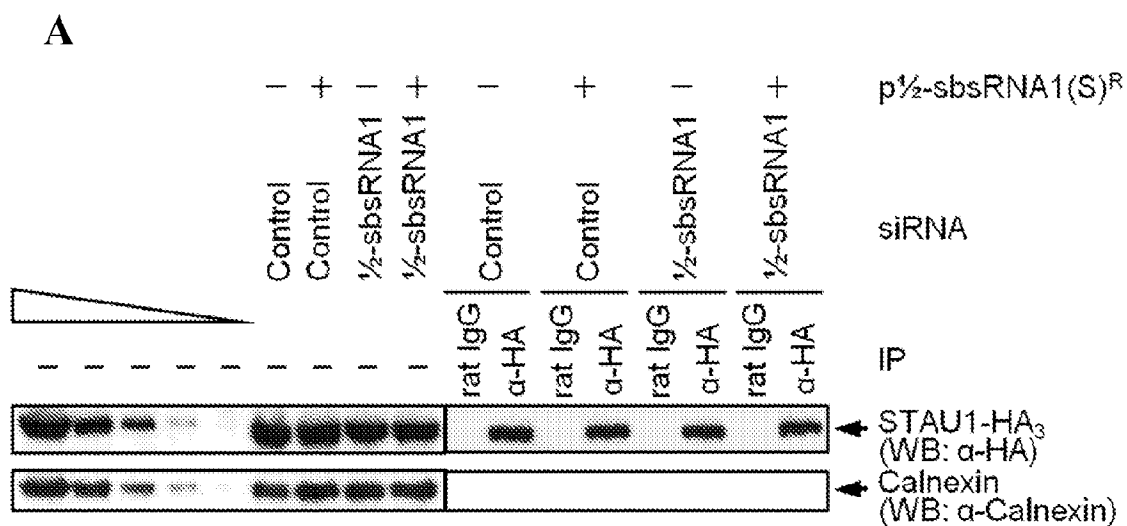
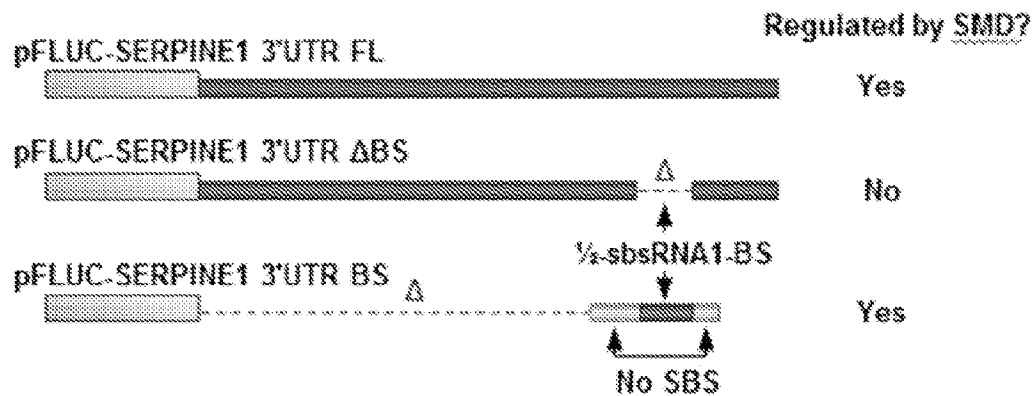


FIG. 6

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B



C

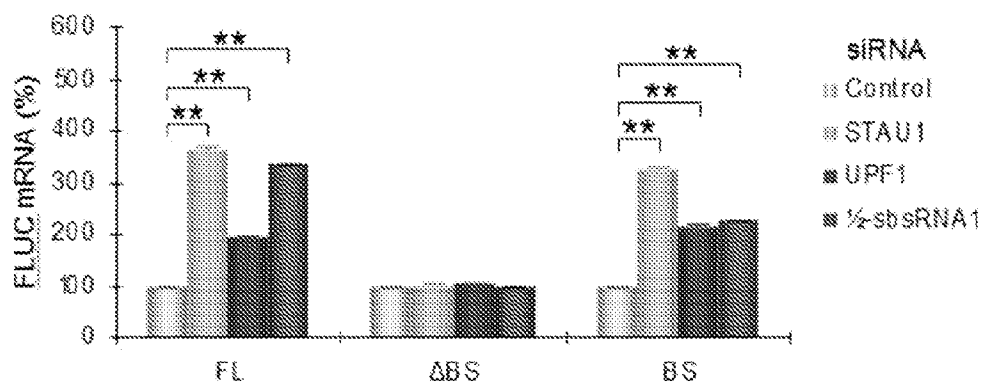
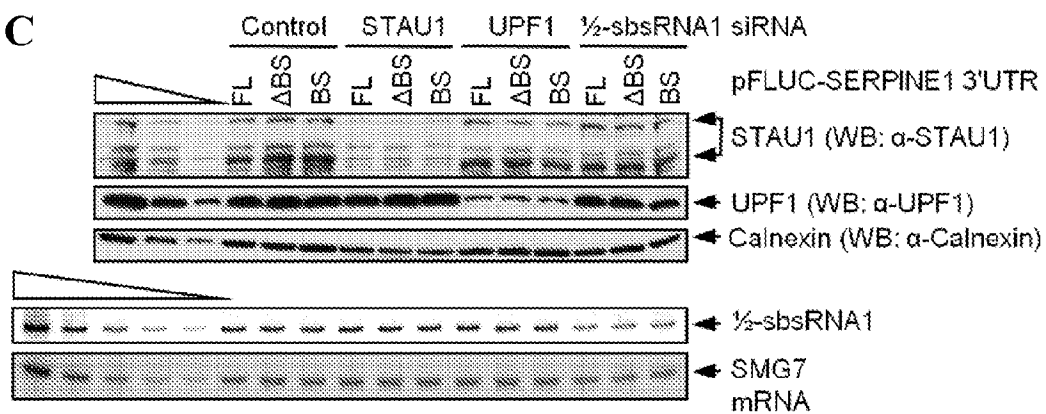


FIG. 6

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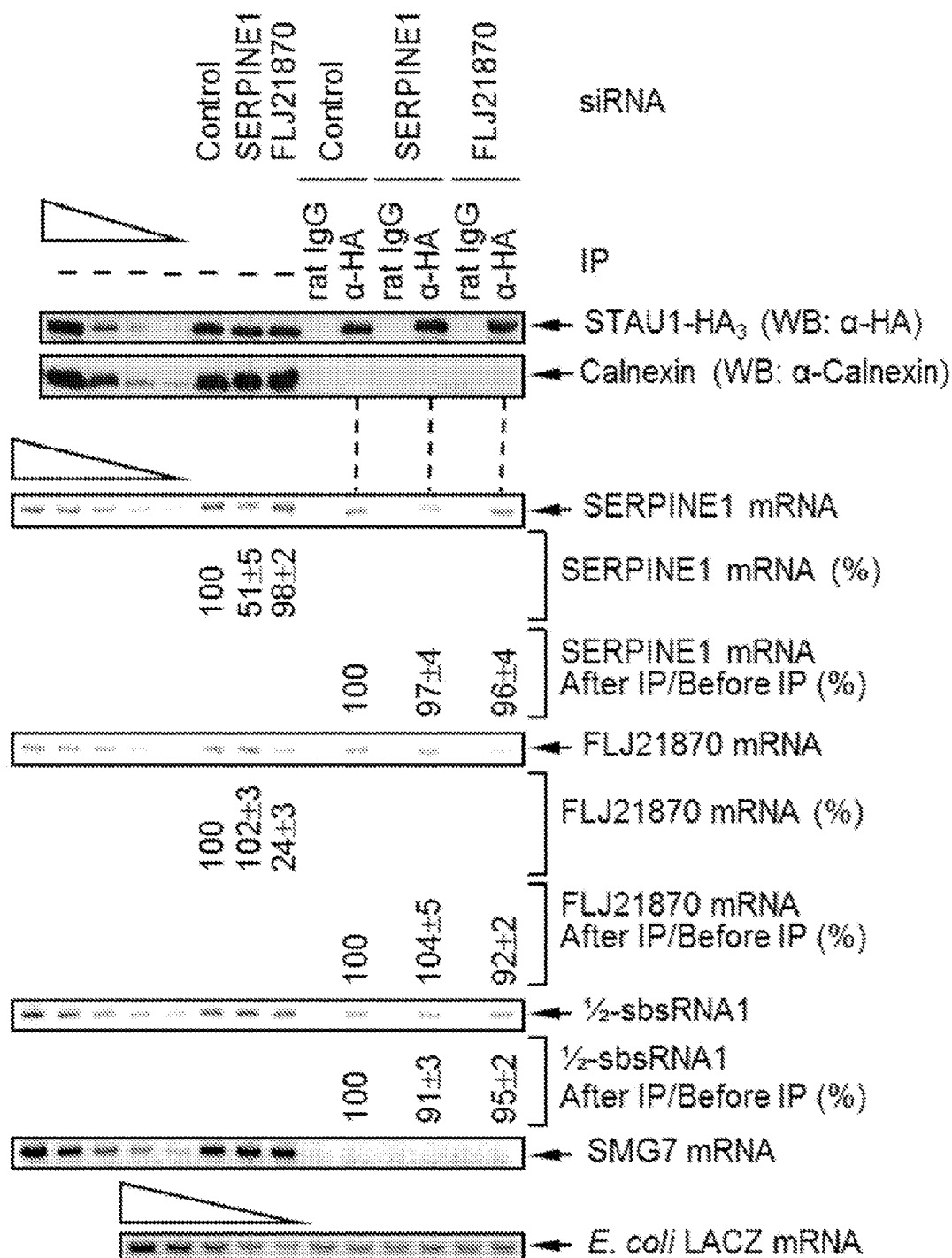


FIG. 7

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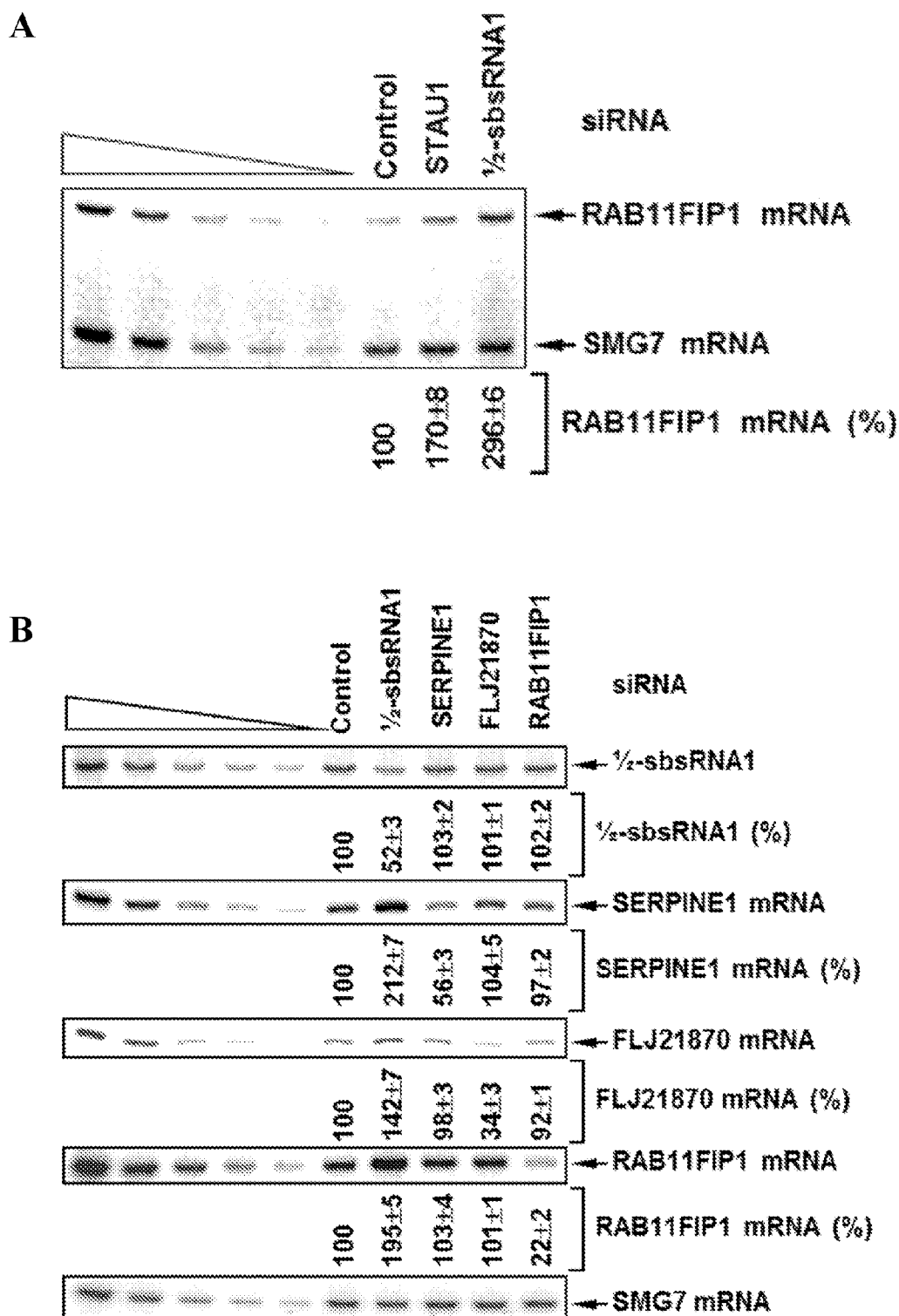


FIG. 8

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C

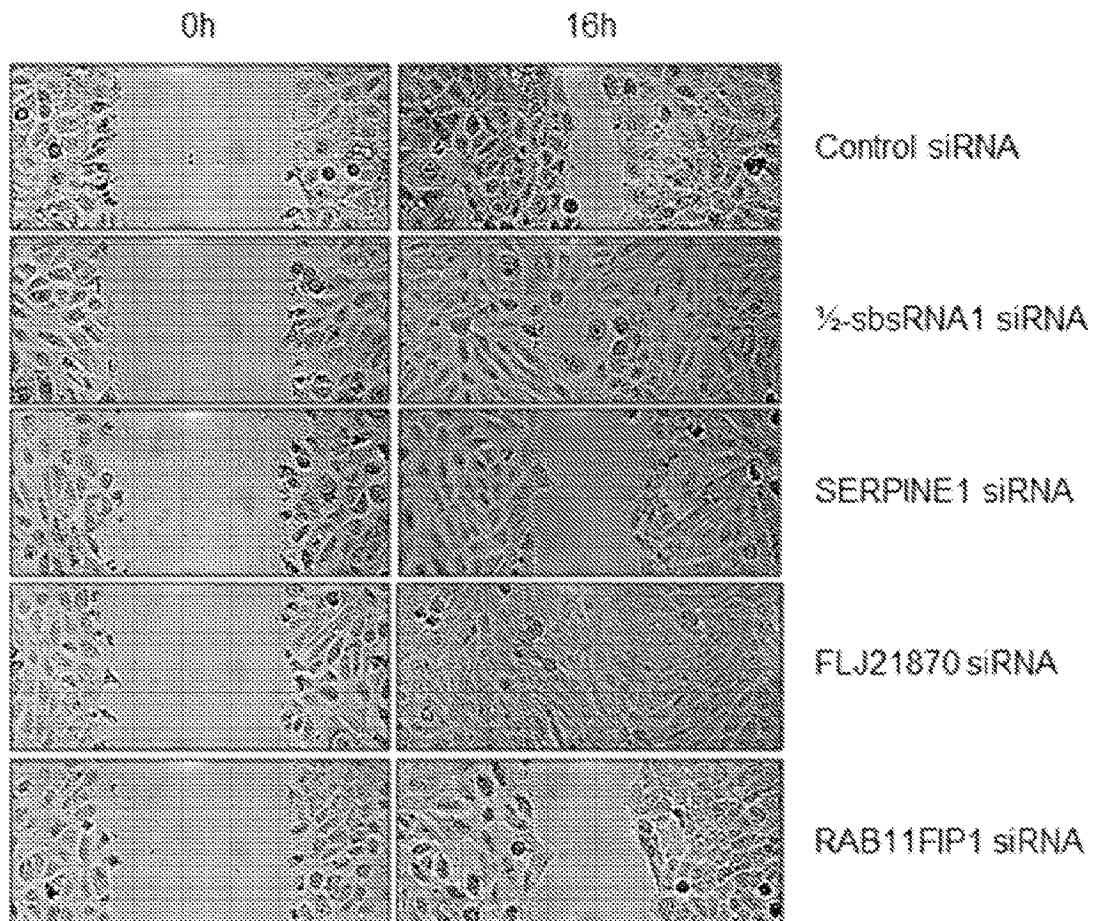


FIG. 8

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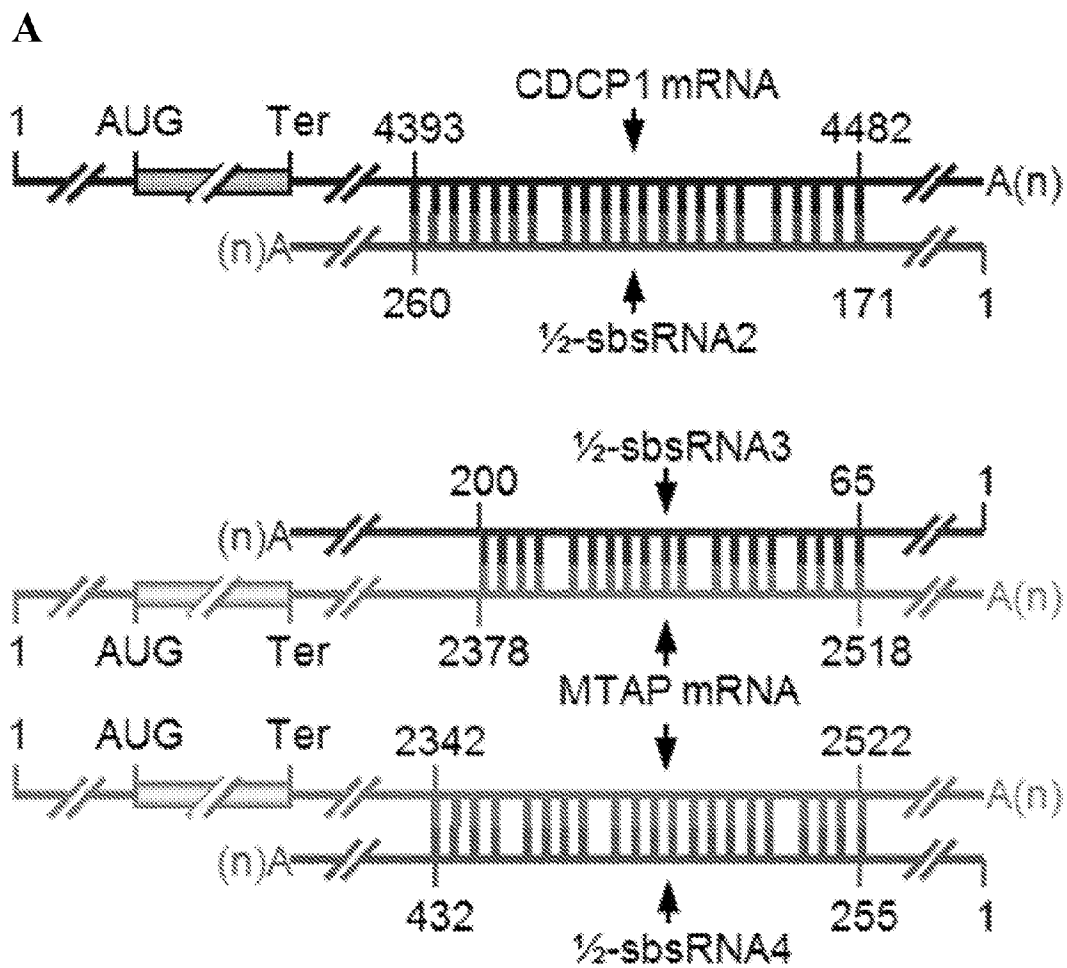


FIG. 9

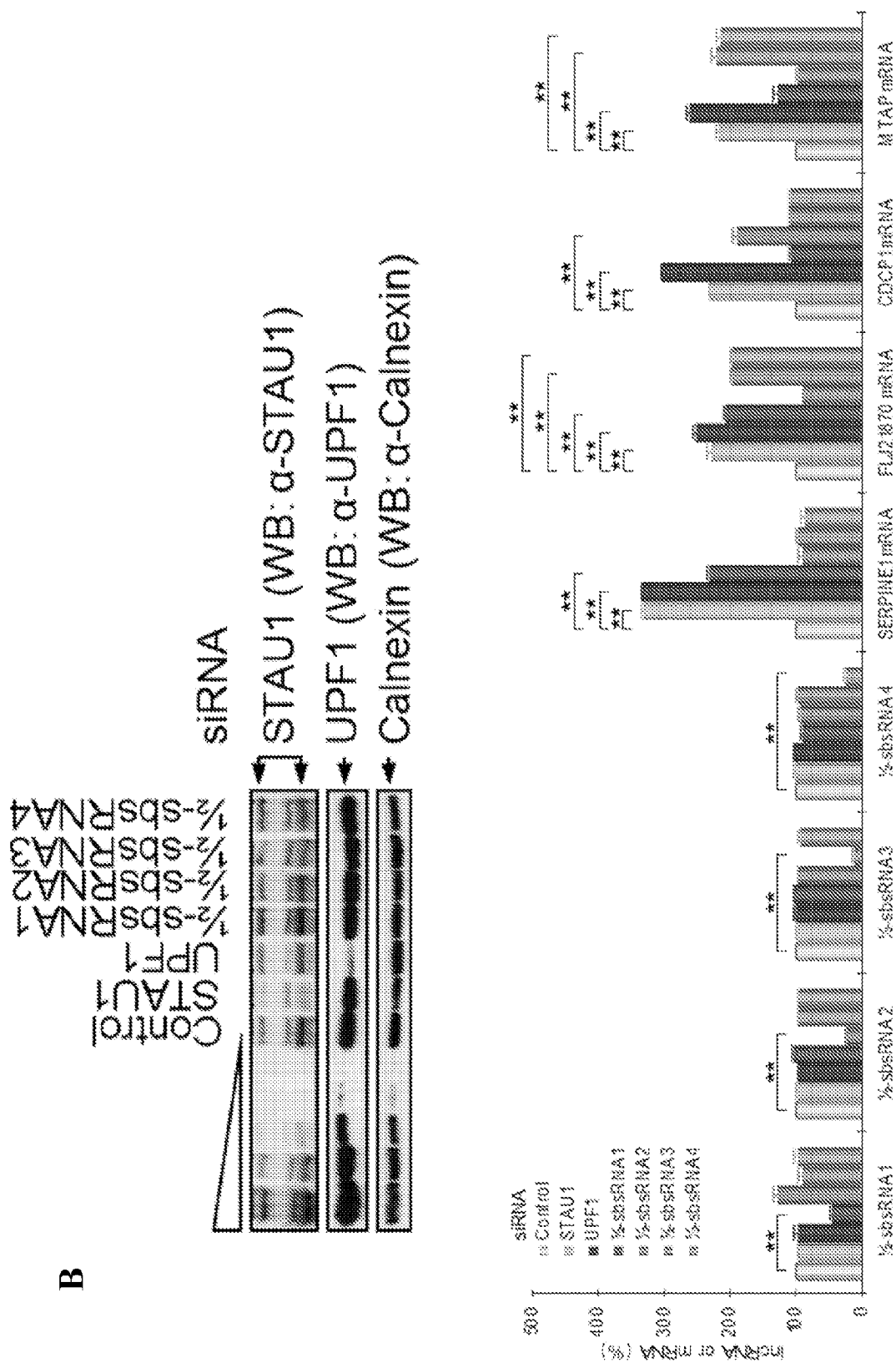


FIG. 9

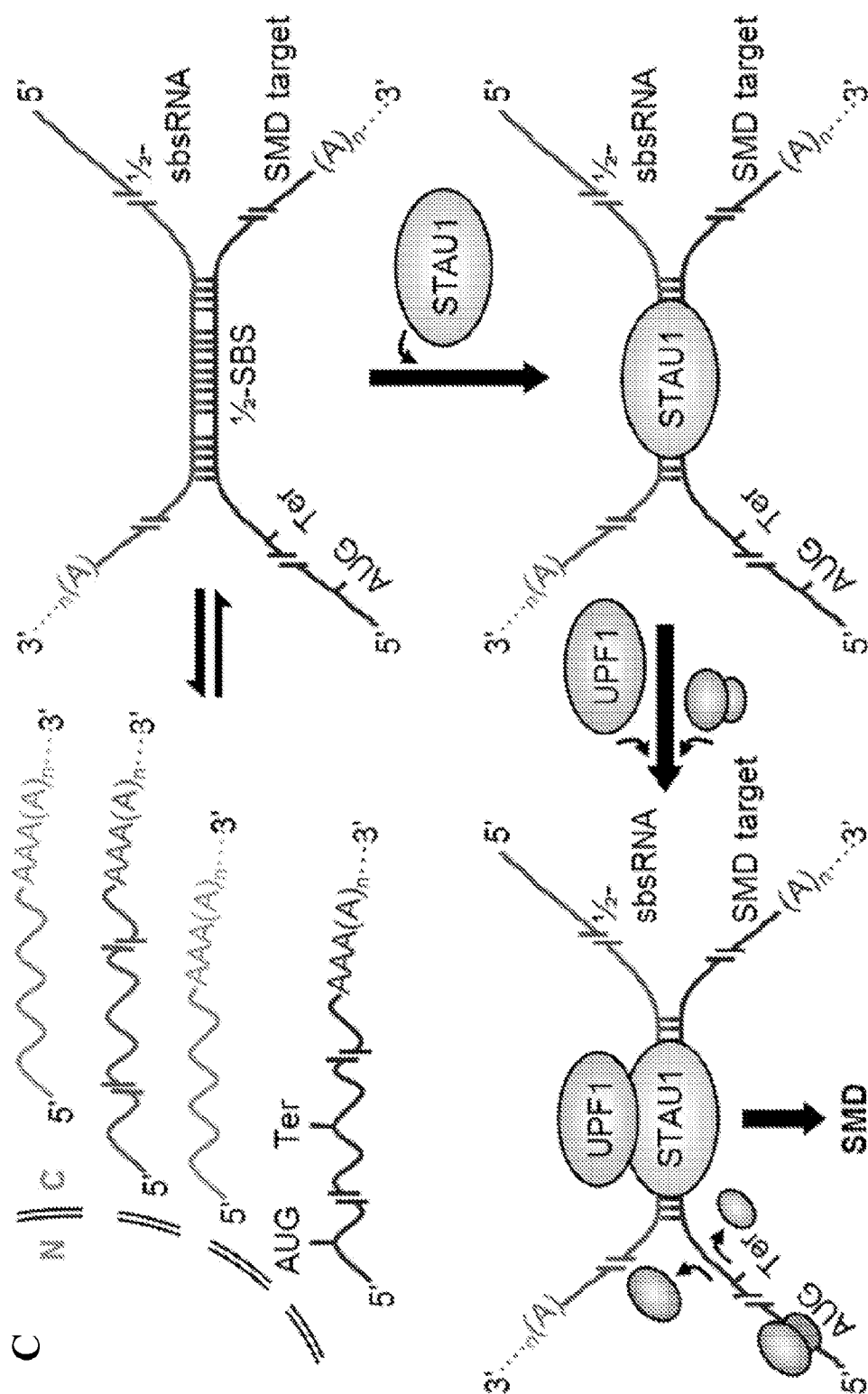


FIG. 9

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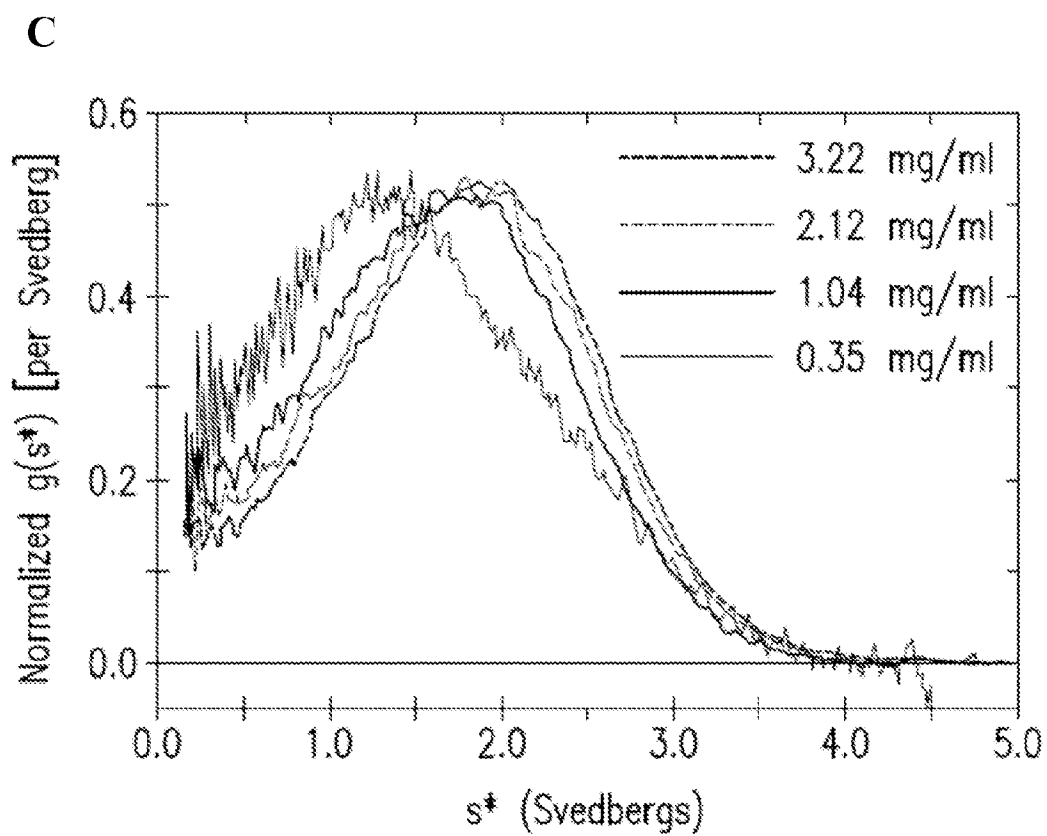
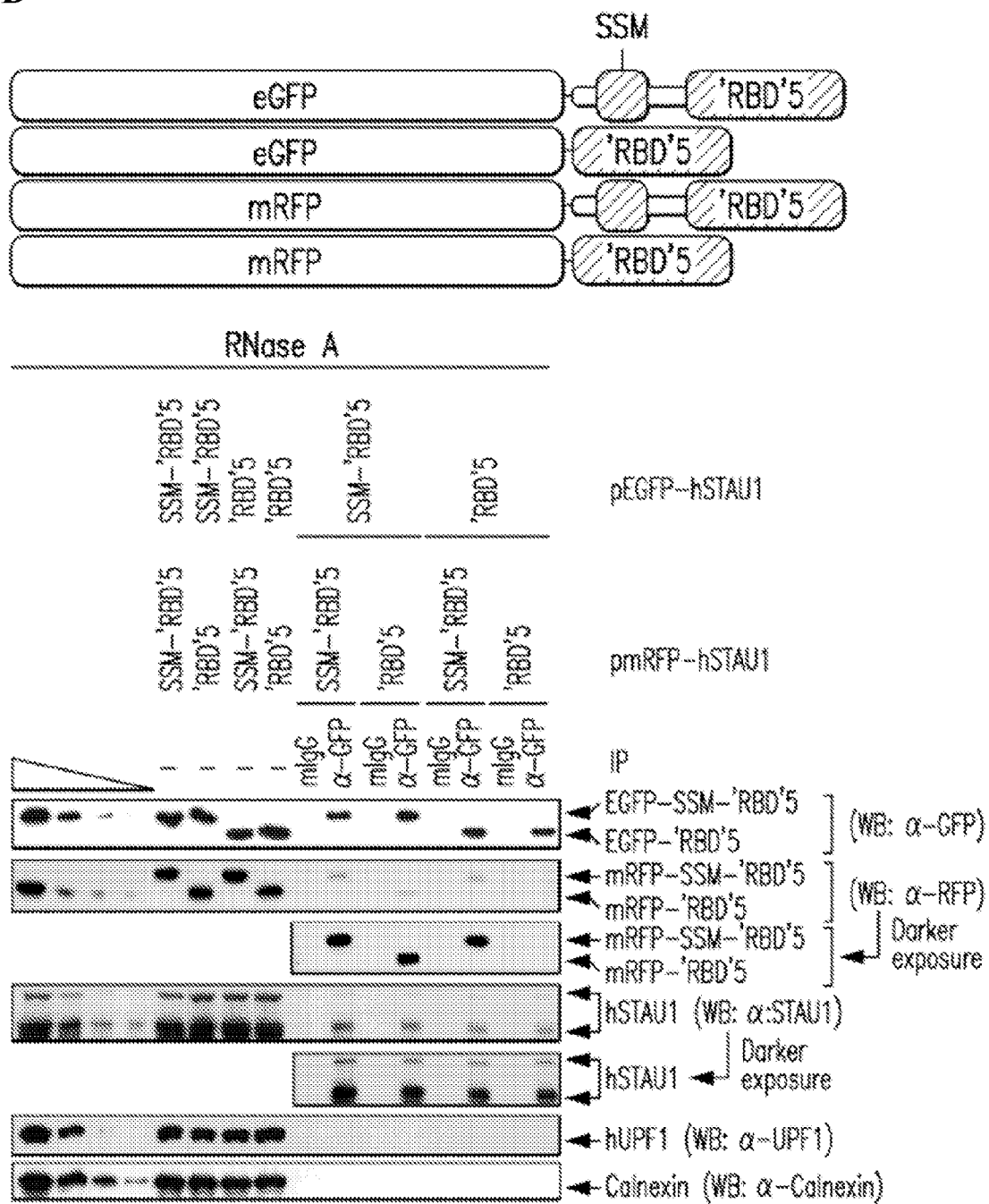


FIG. 10

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D**FIG. 10**

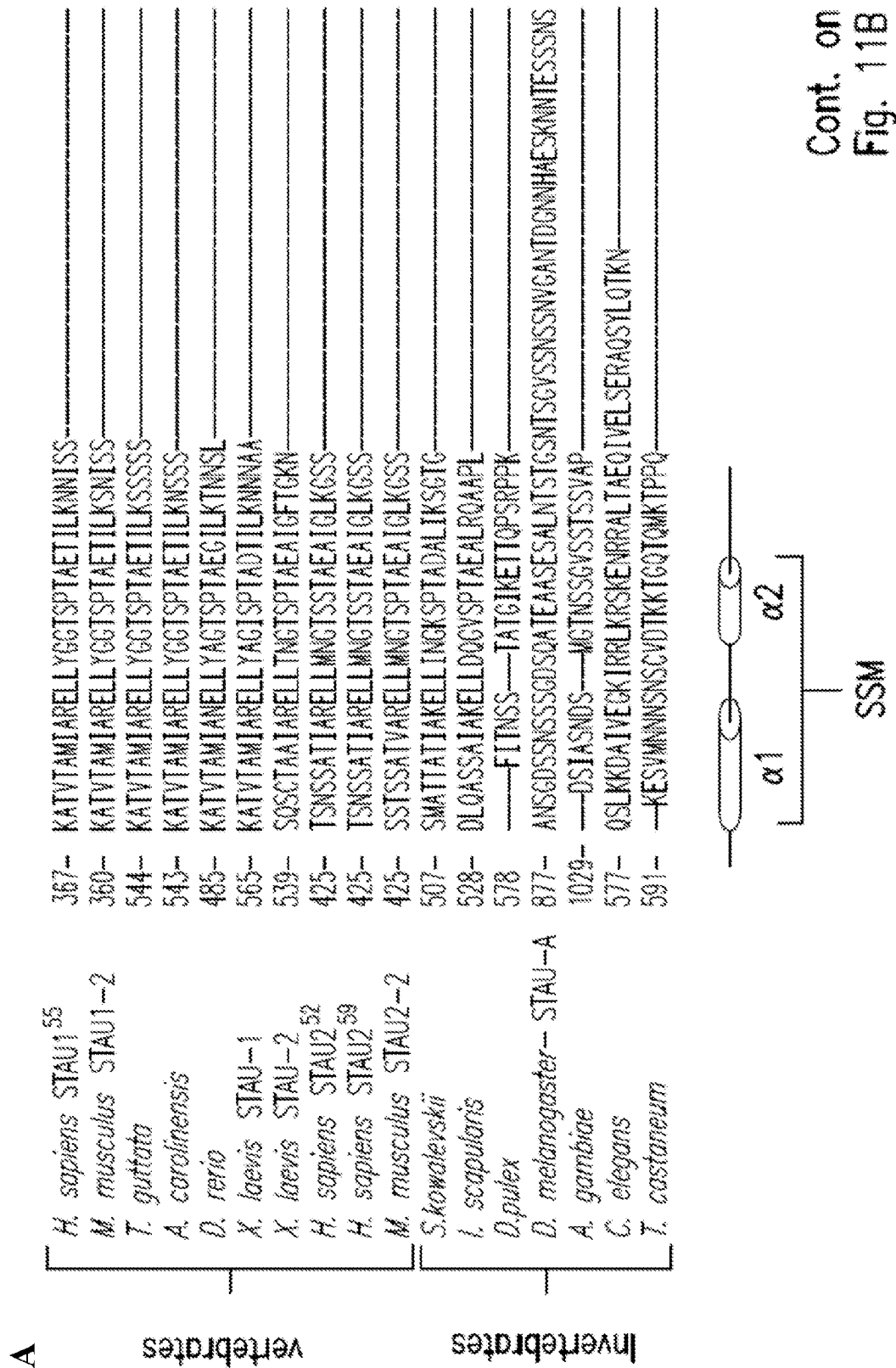


FIG. 11

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B

CHVPHGPLTRPSEQLDYL SRVQCFQVEYKDFPKNKNKNEFVSLINCSSQPLISHGICKOVESCHDMAALNLIKLLSELQDQ -478
 CHVPHGPRTRPSEQLYLSRAQCFQVEYKDFPKNKNKNECVSLINCSSQPLVSHGICKOVESCHDMAALNLIKLLSELQDQ -471
 CHVPHGPLTRPSEQLDYL SNVQGIQVEYKDFPKNKNKNEFVSLINCSSQPLISHGICKOVESCHDMAALNLIKLLSELQDQ -655
 CHVPHGFTTRPSEQLNLSGVQGIQVEYKDFPKNKNKNEFVSLINCSSQPLISHGICKOVESCHDMAALNLIKLLSELQDQ -653
 AHRPQGPLTRPSEQLSYLANVQGIQVEYKDFPKNKNKNEFVSLINCSSQPLISHGICKOVESCHDMAALNLIKLLSELQDQ -596
 CHVLRGFTTRPSEQLNVLARVQGLEVEYKDFPKNKNKNEFVSLINCSSQPLISHGICKOVESCHDMAALNLIKLLSELQDQ -676
 LMCHSSTVQPSKQLEYLAGIQCFQVNYSDRQNGNDFLCLILSPVQ---MIFHGIGSSLEASHDQAALSALKQFSEQGLE -645
 -PTPPCSPVQPSKQLEYLARIQCFQV -479 (C-terminus)
 -PTPPCSPVQPSKQLEYLARIQCFQA-----ALSALKQFSEQGLD -493
 -PTSPCSPVQPSKQLEYLARIQCFQA-----ALSALKQFSEQGLE -493
 -PKTEQKLLRPKQQLMFLAEIQGLQVEFTDFPKGNKPEYLSVSIATNPPLVAHGACPTVDSYDAAALQALKSLTDMDIG -616
 ---TVAPVPRPKQIILYLAEVLGFQVHFTDFPKGNKROFLSLVLTTCPPQVSHGAGSSLEASHDEAALAVLRTLARRGLD -634
 ---TSGGLRPEIRLRYLAQVLEYEVEFSDFPKGKKGFVSLVLTNPVQVSHGCGATLEEAHDMAASNMKLLSECSQD -676
 TSNTQSAGVHWKEQLLYLSKLLDFEVNFSQPKGNHNEFLTIIVLSTHPPQICHGVCKSSEESQNDAA SNALKILSKLGLN -1022
 ---KADIGAKKEQLLYLAQLLKFEVQFSDFPKGNHGEYLTIVLSTIEPPQLCHGSCASLOESHDEAARGALEILSKIG-L -1128
 TTIQSSQSSAHHLEQLSDFFKFSLOYTSFPQVGIQCHFTIIVSIGLEAPLYGHGTCGSTTEADENAALDAIAKLKEL SAS -703
 ---PTQGVRSKDQLMYIAQLMNIQVQFSDFPKANHEMYLTIVLSLTNPVQVCHGCEGPTTEASHEKAALKVLSELG-L -694

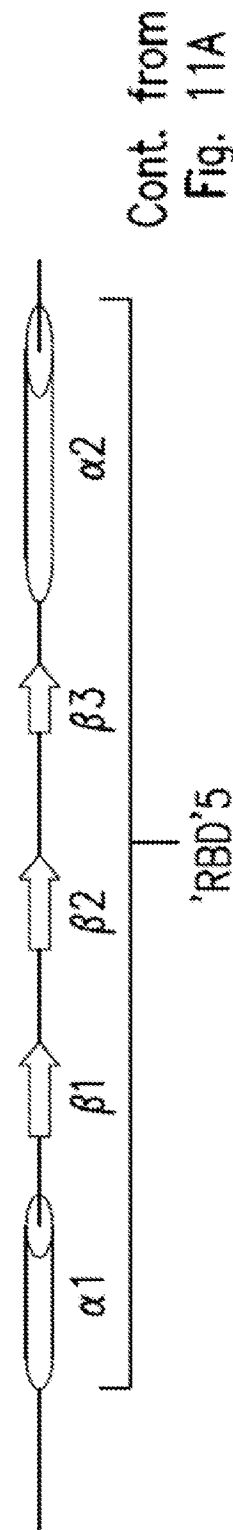


FIG. 11

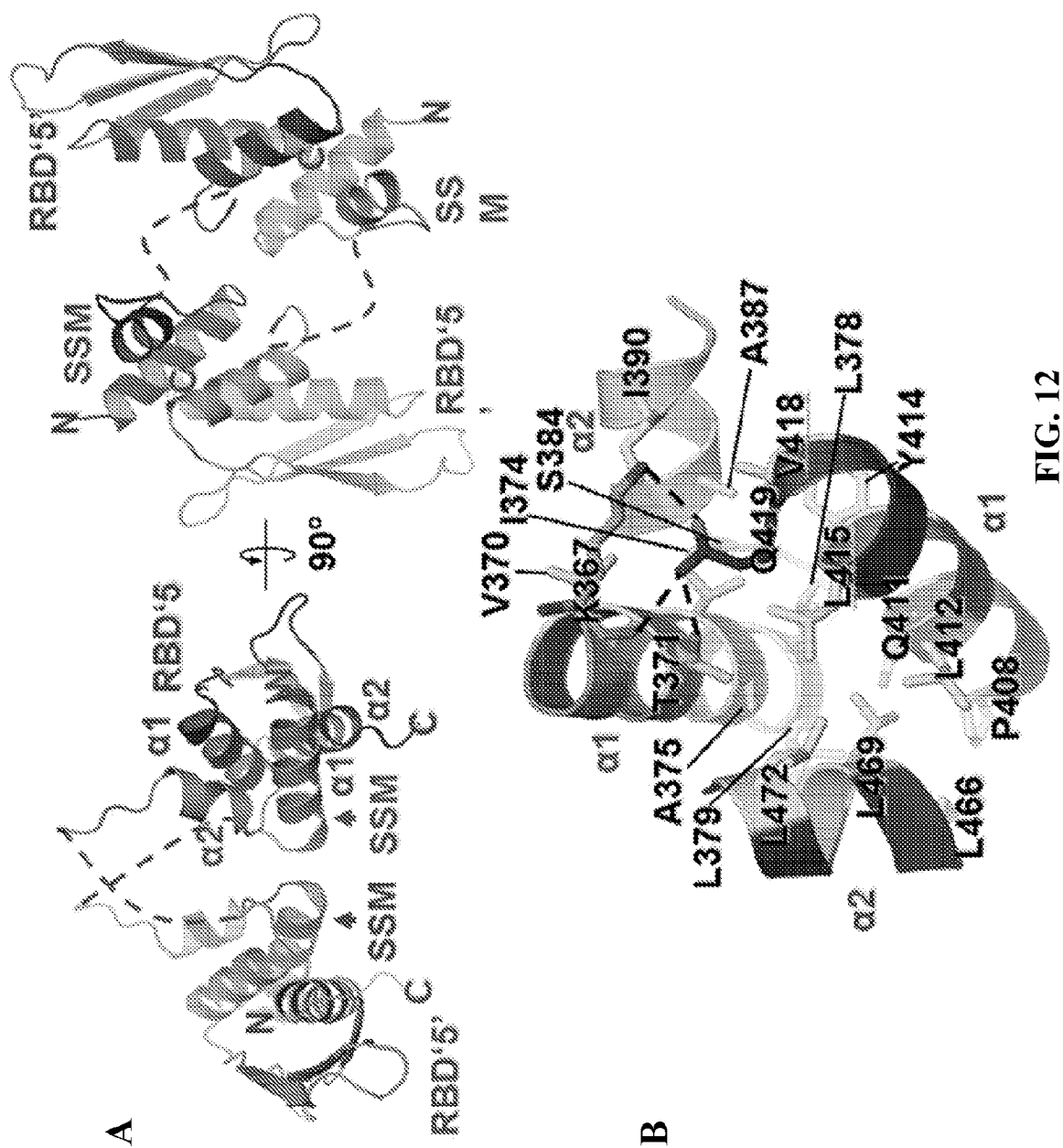


FIG. 12

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A-1

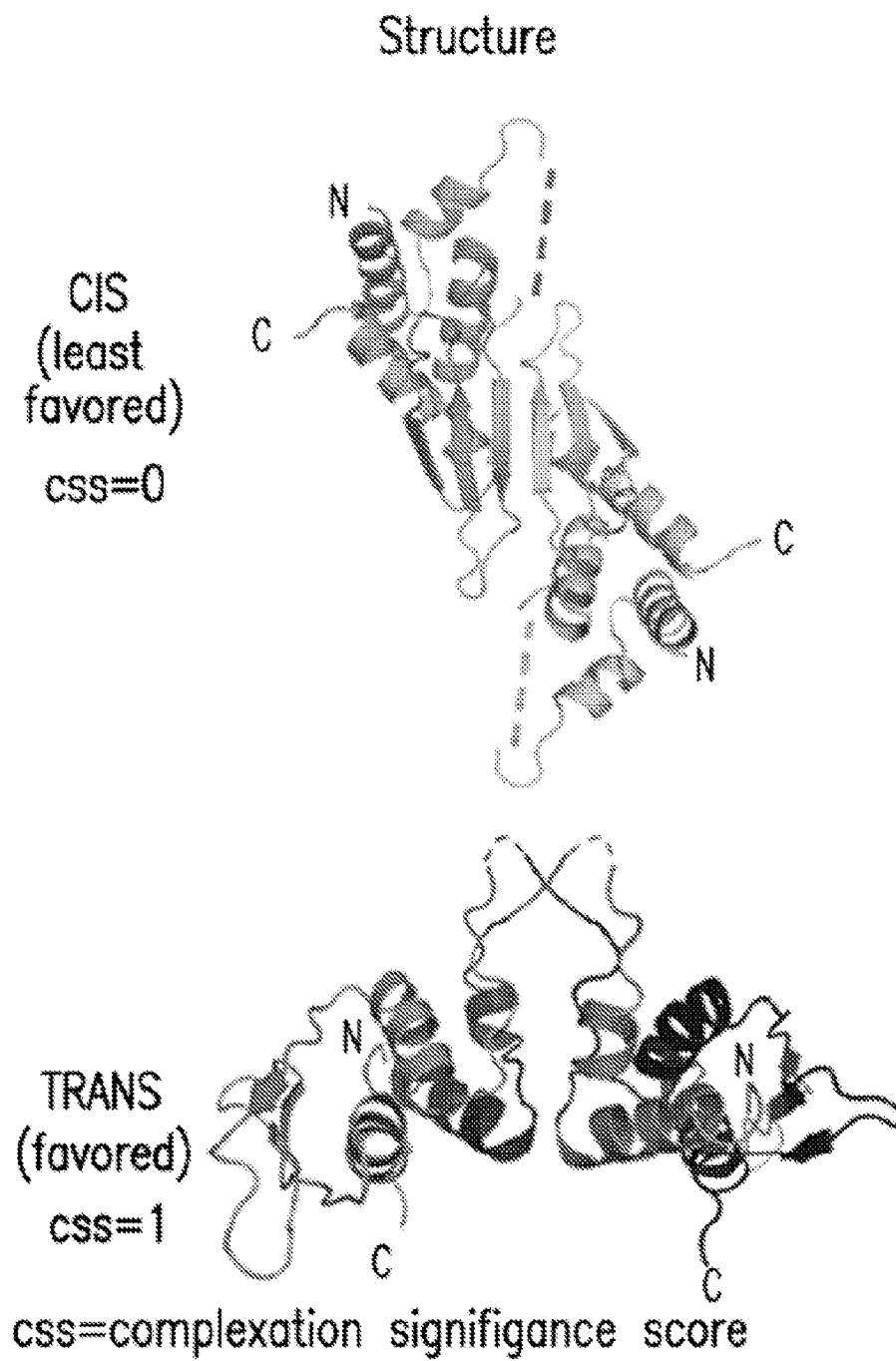


FIG. 13

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A-2

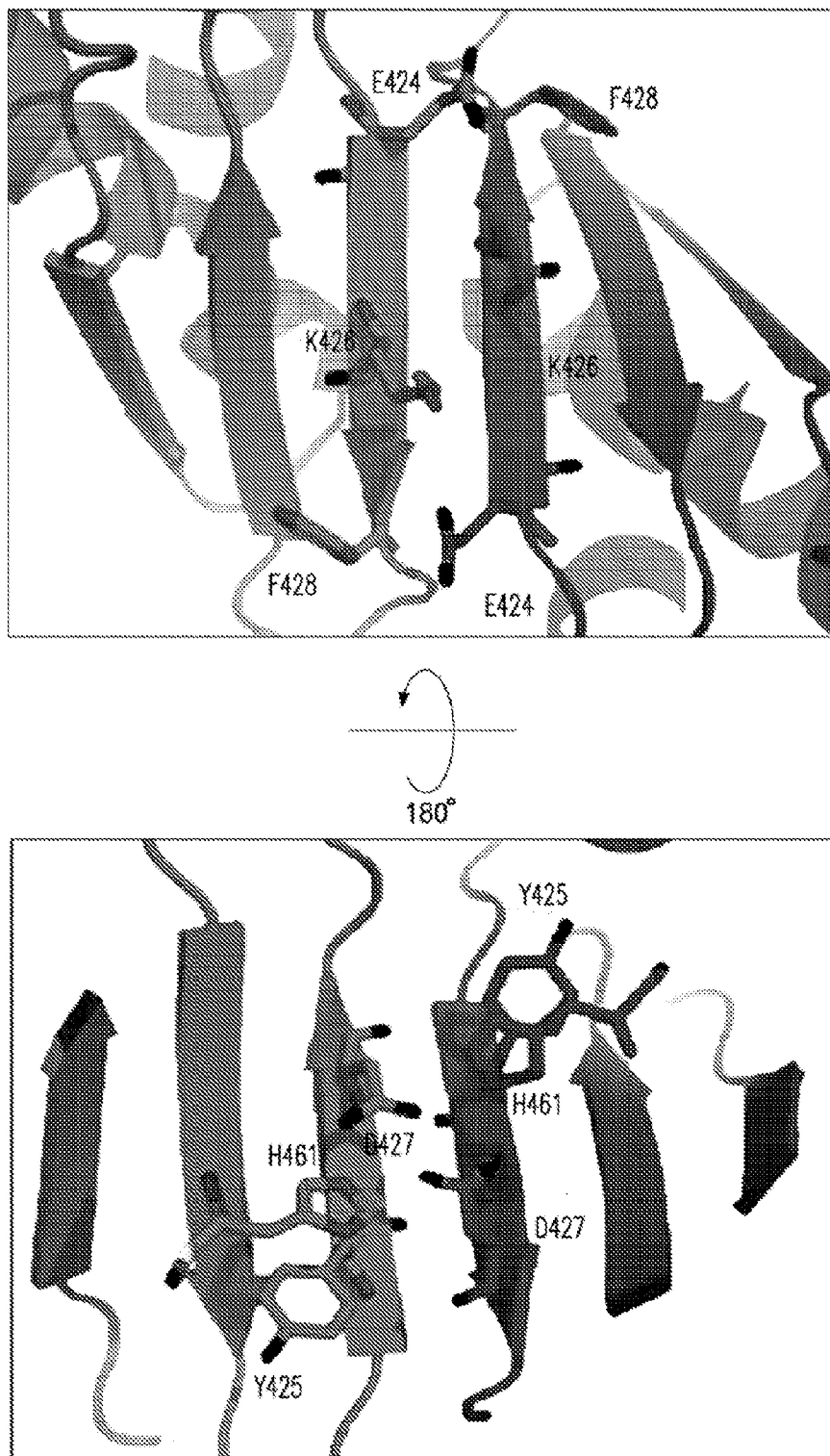
PISA Result

	Structure 1		Structure 2	
Selection range	A		A	
Class	Protein		Protein	
Symmetry operation	x,y,z		y,x,-z	
Symmetry ID	1_555		7_555	
Number of atoms				
interface	80	(9.8%)	82	(10.0%)
surface	505	(61.7%)	505	(61.7%)
total	818	(100.0%)	818	(100.0%)
Number of residues				
interface	22	(20.8%)	22	(20.8%)
surface	97	(91.5%)	97	(91.5%)
total	106	(100.0%)	106	(100.0%)
Solvent-accessible area,A ²				
interface	763.8	(11.3%)	764.4	(11.3%)
total	6736.3	(100.0%)	6736.3	(61.7%)

	Structure 1		Structure 2	
Selection range	A		A	
Class	Protein		Protein	
Symmetry operation	x,y,z		-y+1,-x+1,-z+1/2	
Symmetry ID	1_555		8_665	
Number of atoms				
interface	128	(15.5%)	124	(15.0%)
surface	565	(68.5%)	565	(68.5%)
total	825	(100.0%)	825	(100.0%)
Number of residues				
interface	38	(35.5%)	37	(34.6%)
surface	104	(97.2%)	104	(97.2%)
total	107	(100.0%)	107	(100.0%)
Solvent-accessible area,A ²				
interface	1248.2	(15.4%)	1249.7	(15.4%)
total	8101.7	(100.0%)	8101.7	(100.0%)

FIG. 13

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B**FIG. 13**

SUBSTITUTE SHEET (RULE 26)

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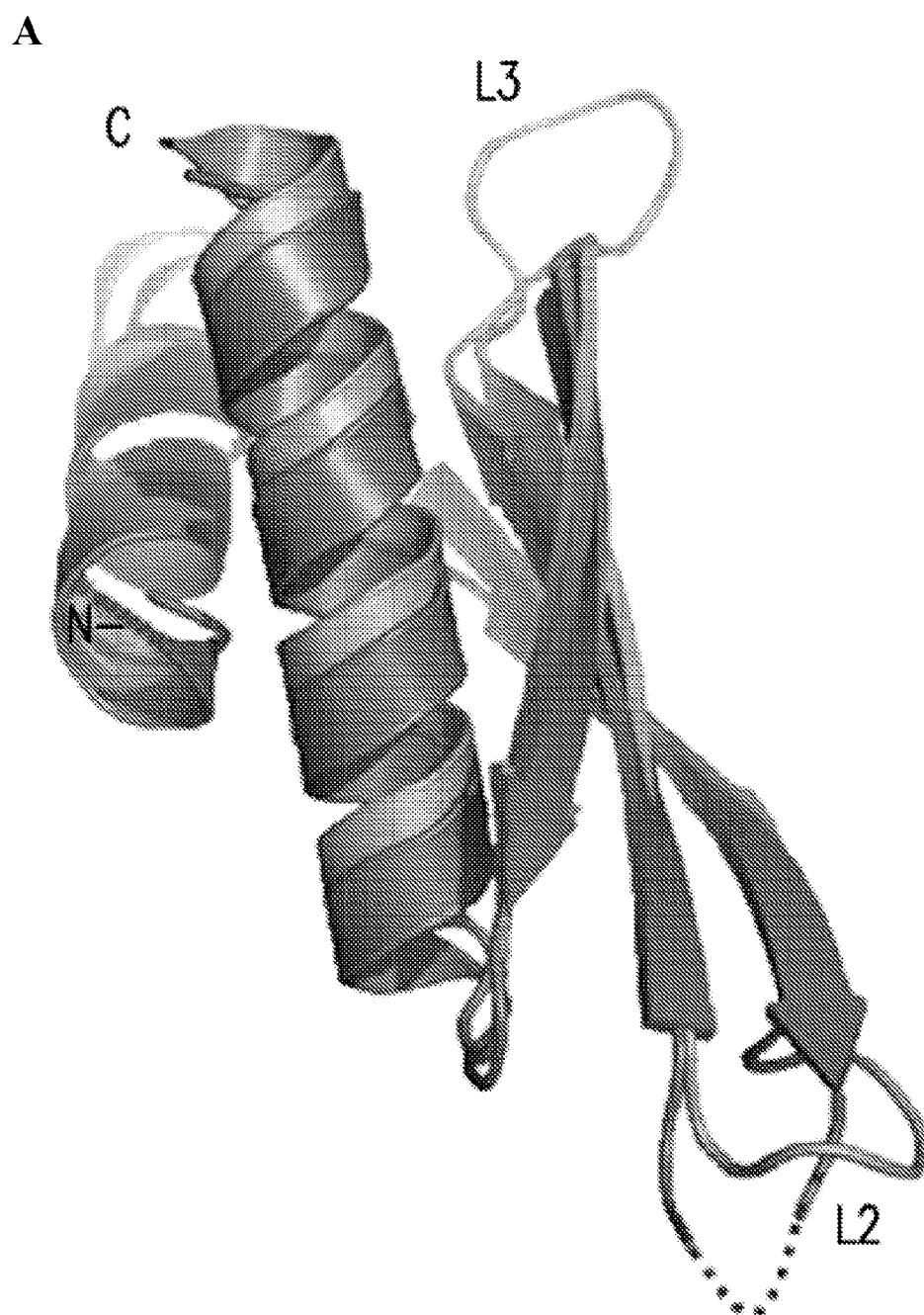


FIG. 14

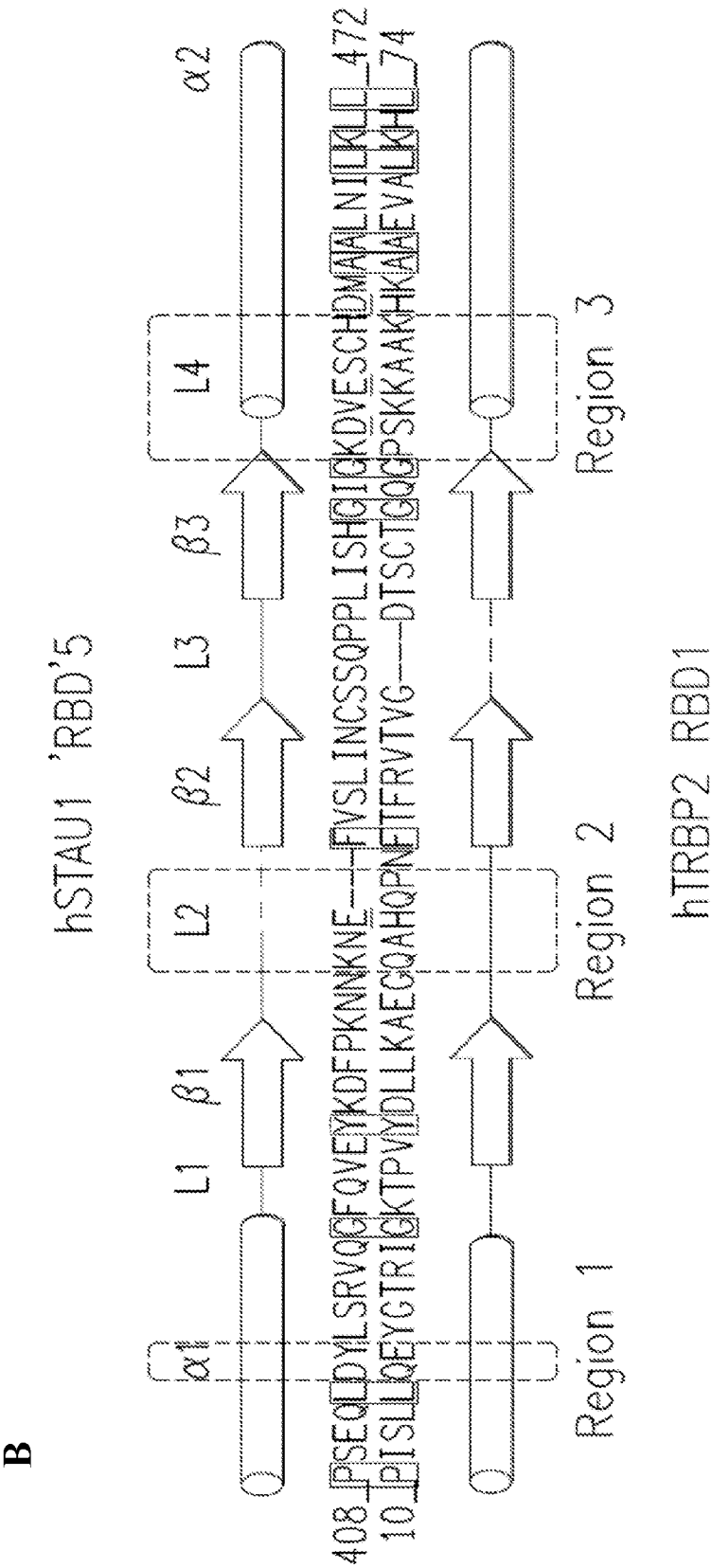


FIG. 14

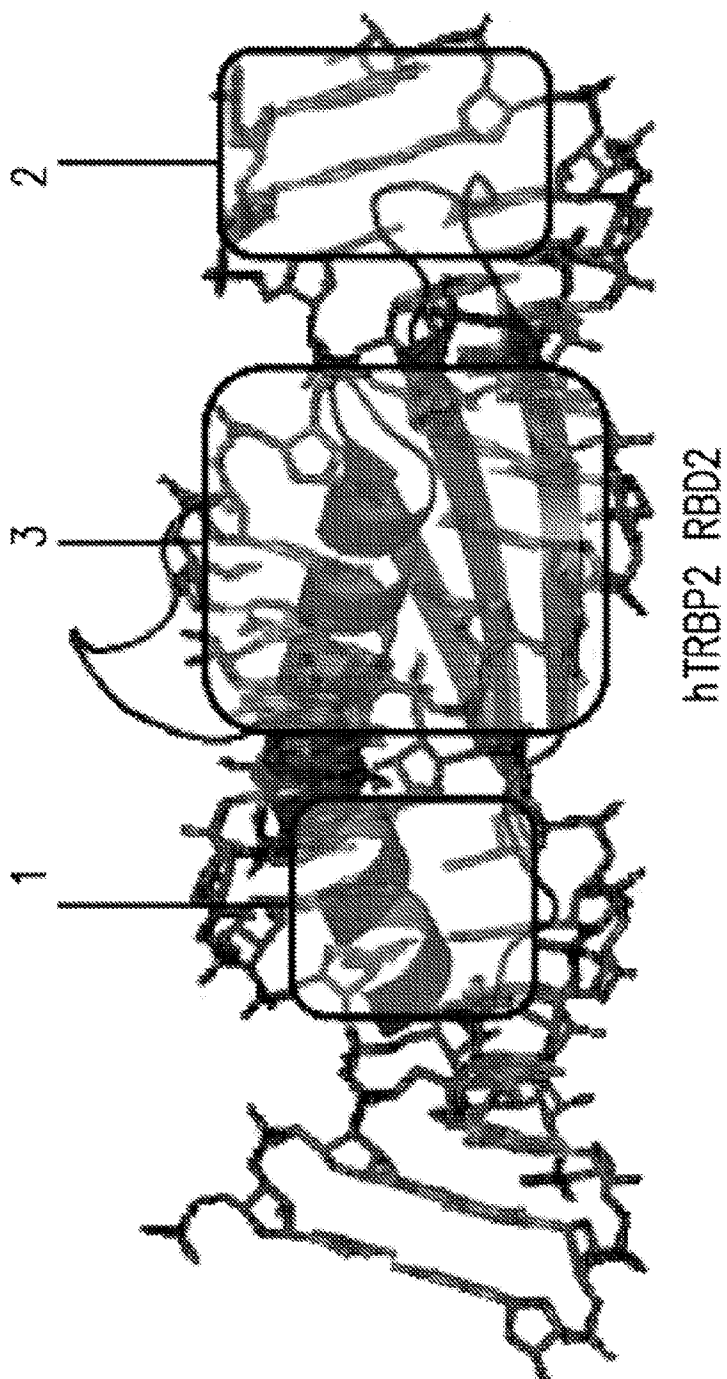


FIG. 14

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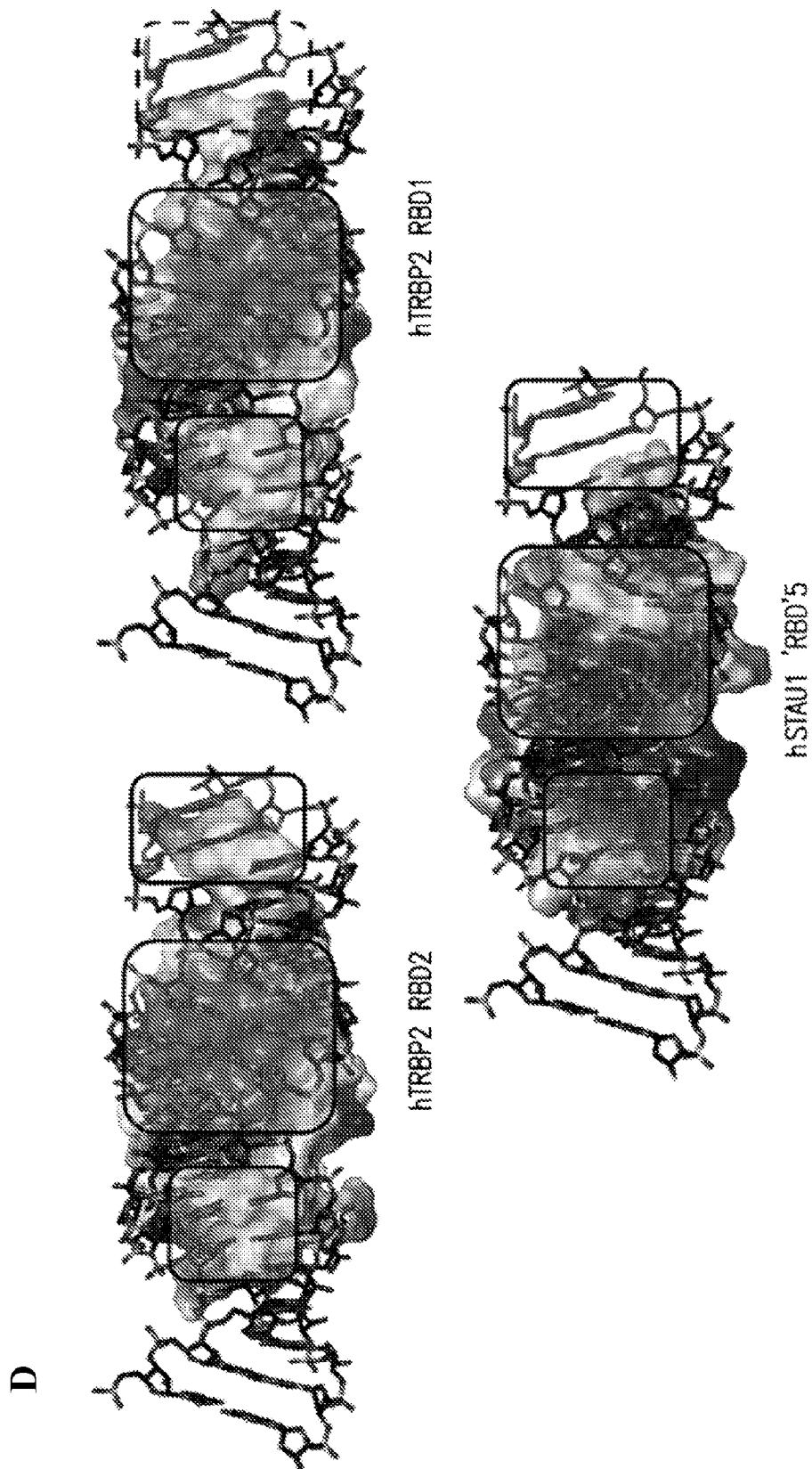


FIG. 14

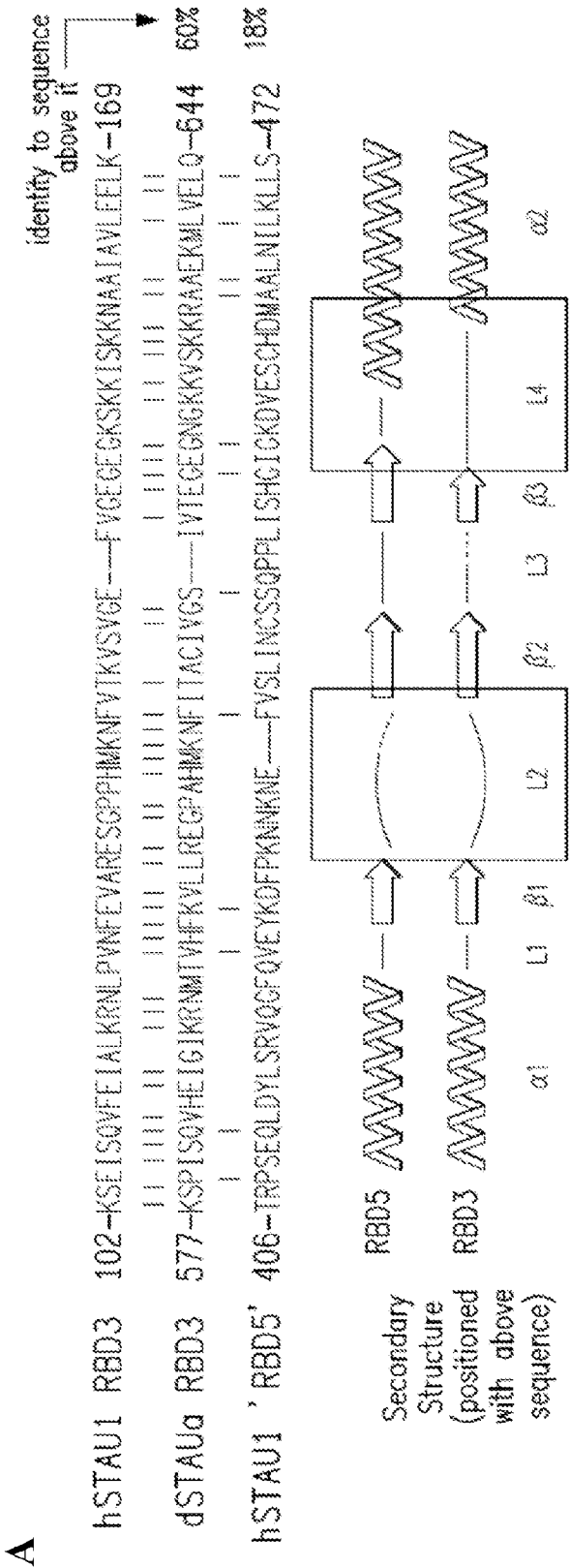


FIG. 15

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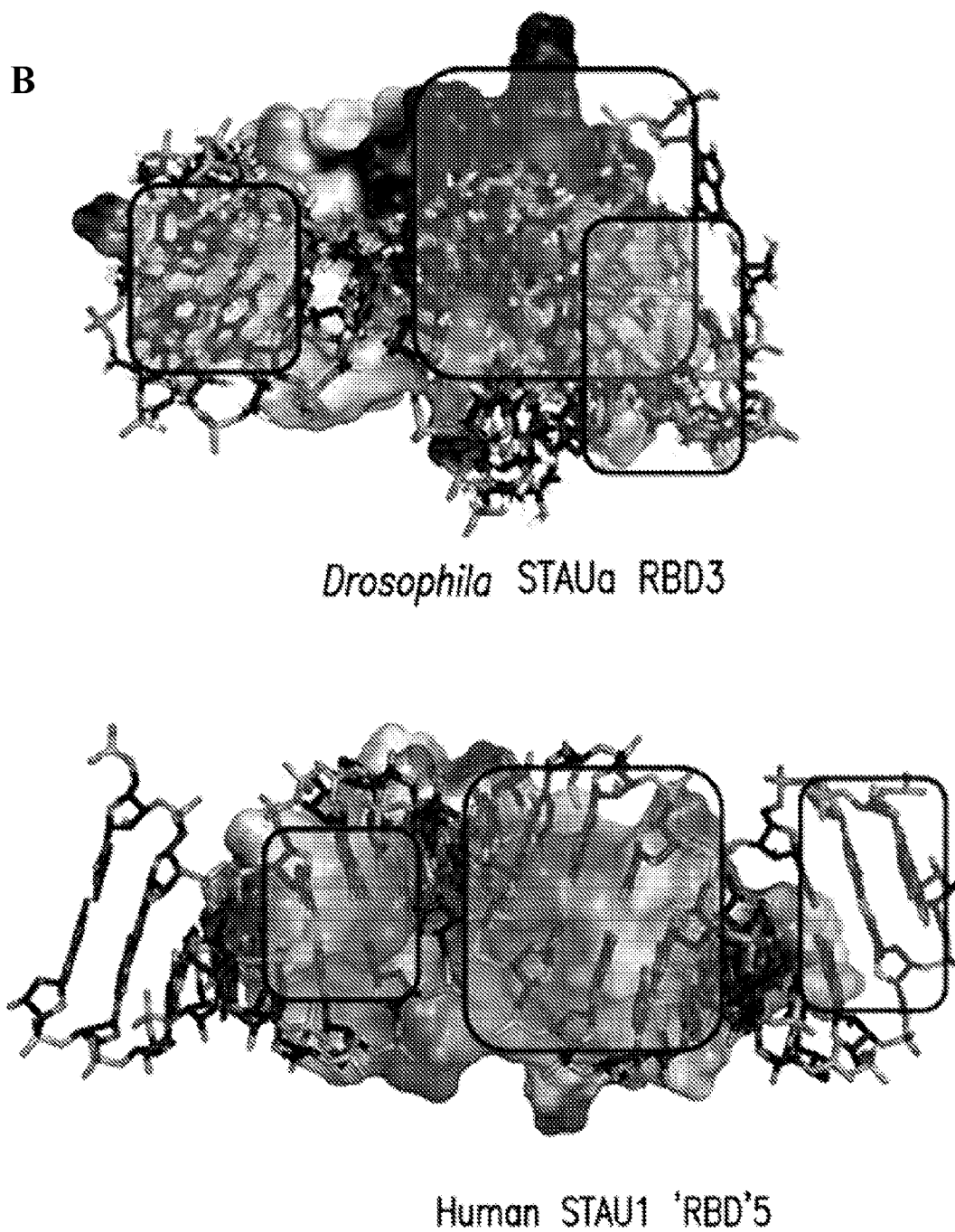


FIG. 15

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/24534

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07H 21/00 (2012.01)

USPC - 536/23.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

USPC -- 536/23.1, 536/24.1, 514/44R, 514/44A

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 PubWEST -- PGPB,USPT,USOC,EPAB,JPAB; Dialog Classic Files -- 654, 652, 349, 348, 340, 35, 65, 155; USPTO Web Page; PCT Patentscope; Entrez Pubmed; Google Scholar; Search terms -- Staufen-1, sbs, mRNA decay, down-regulation, up-regulation, ALU, lncRNA, SMD targets. siRNA, microarray, Western blot, GAP43 target, immunoprecipitation, computer analysis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y -- A	WO 2006/039399 A2 (Maquat et al.) 13 April 2006 (13.04.2006) pg 2, ln 1-2; pg 7, ln 26 -- pg 8, ln 5; pg 9, ln 8-16; pg 11, ln 1-16; pg 13, ln 27 -- pg 14, ln 2; pg 16, ln 19 -- pg 17, ln 9; pg 17, ln 18 -- pg 18, ln 23; pg 24, ln 22-25; ; pg 24, ln 30 -- pg 25, ln 18; pg 60, ln 28 -- pg 61, ln 12; pg 63, ln 4-13; pg 64, ln 40 -- pg 66, ln 11; pg 65, ln 23-27, Figs 2, 9, 15, 20	1-5 ----- 6-8
Y -- A	CHIU, Y.L et al. High-molecular-mass APOBEC3G complexes restrict Alu retrotransposition. Proc. Natl. Acad. Sci. USA, 17 October 2006, Vol 103, No. 42, pages 15588-15593; pg 15990, col 2, para 3; pg 15992, col 1, pra 1; col 2, para 1, Fig 1D; pg 15990, col 2, para 3	1-5 ----- 6-8
A	HUNG, T. et al. Long noncoding RNA in genome regulation Prospects and mechanisms. RNA Biology, Sept/Oct 2010, Vol. 7, No. 5, pages 582-585; pg 582, col 2, para 1-2	6-8
P/X	GONG, C. et al. lncRNAs transactivate Staufen1-mediated mRNA decay by duplexing with 3'UTRs via Alu elements. Nature, 10 February 2011, Vol. 470, Pages 284-288; pg 284, para 2, abstract; pg 285, para 2,5; pg 286, para 1; pg 288, para 3 (note: author's manuscript uploaded for the purposes of this ISR).	1-7

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

11 July 2012 (11.07.2012)

Date of mailing of the international search report

27 JUL 2012

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/24534

Continuation of Box III

Group III claims 10-13 directed to a method of screening for an agent that modulates SMD comprising incubating the agent with a stably transfected cell comprising a reporter gene with a 3' UTR ALU element; a lncRNA containing a corresponding ALU element; and Stau 1, and assaying the amount of mRNA of the reporter gene in the cell, wherein an increase or decrease in the amount of mRNA of the reporter gene relative to the amount of mRNA of the reporter gene in the absence of the agent indicates a substance that modulates SMD activity.

Group IV claims 14-17 directed to a method of screening for an agent that modulates SMD comprising incubating the agent with an SMD target and Stau1 forming a substanceSMD target-Stau1 mixture, and assaying the amount of SMD target-Stau1 complex present in the mixture, wherein an increase or decrease in the amount of SMD target -Stau1 complex relative to the amount of SMD target -Stau 1 complex in the absence of the agent indicates that the substance promotes or inhibits SMD, respectively.

Group V claims 18-21 directed to a method of screening for an agent that modulates SMD comprising incubating the agent with an SMD target and Stau1 forming a substanceSMD target-Stau1 mixture, and assaying the amount of SMD target present in the mixture, wherein an increase or decrease in the amount of SMD target relative to the amount of SMD target in the absence of the agent indicates that the substance inhibits or promotes SMD, respectively.

Group VI claims 22-26 directed to an agent that modulates SMD.

Group VII claims 27-28 directed to a method of identifying an agent that binds an SBS.

Group VIII claims 29-33 directed to a method of identifying genes modulated by the down-regulation of an ALU element containing lncRNA.

Group IX claims 34-46 directed to a method of treating a disorder associated with expression of a truncated or erroneous protein in a subject.

The inventions listed as Groups I-IX do not relate to a single inventive concept under Rule 13.1 because under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical features of each Group are delineated above. The shared technical feature of Groups I-IX are methods and an agent involving aspects of Stau1-mediated mRNA decay (SMD). However, this is not an improvement over the prior art of WO 2006/039399 A2 to Maquat et al. (hereinafter "Maquat") that teaches methods and agents involving Stau1-mediated mRNA decay (SMD) (abstract; pg 16, ln 19 to pg 17 ln 9; pg 17 ln 18-22). Group I has the further special technical features of down-regulating Stau1 or an ALU element-containing long noncoding RNA (lncRNA); identifying transcripts that are up-regulated at least 1.8-fold upon downregulation of Stau1 or the ALU element-containing lncRNA, wherein transcripts that are up-regulated at least 1.8-fold are SMD targets; and identifying an ALU element within the 3' UTR of the transcript of the SMD target, wherein the ALU element comprises a first strand of the SBS. The special technical feature of Group I of down-regulating Stau1 or an ALU element-containing long noncoding RNA (lncRNA) and identifying transcripts that are up-regulated at least 1.8-fold upon downregulation of Stau1 or the ALU element-containing lncRNA, wherein transcripts that are up-regulated at least 1.8-fold are SMD targets are not specifically shared by any of the other groups. Likewise this is not an improvement over the prior art of Maquat that teaches down-regulating Stau1 (Example 3 pg 60 ln 28- pg 61 ln 12; pg 63 ln 4-13; Fig 2, Fig. 9) and identifying transcripts that are up-regulated at least 1.8-fold upon downregulation of Stau1 or the ALU element-containing lncRNA, wherein transcripts that are up-regulated at least 1.8-fold are SMD targets (pg 66 ln 10-11 "the level of Arf1 mRNA that derived from cDNA harboring a nonsense codon at position 35 was up-regulated 2-fold when Stau1 was down-regulated"). Likewise, the special technical feature of Group I of identifying an ALU element within the 3' UTR of the transcript of the SMD target, wherein the ALU element comprises a first strand of the SBS is not specifically shared by any of the other Groups. Further, this special technical feature does not represent an improvement over the prior art of the article entitled "High-molecular-mass APOBEC3G complexes restrict Alu retrotransposition," by Chiu et al. (hereinafter "Chiu") teaches recruitment (binding) of ALU transcripts into Staufen1 (Stau1) granules (pg 15591, Fig 1D; pg 15592, col 1, para 1, col 2, para 1). Although Chiu does not specifically teach the localization of the SBS on the ALU element as a first strand of the SBS, it would have been inherent that the interaction of Stau1 and the ALU sequence taught by Chiu involves a region on a strand of sequence complementarity and further that the site encompasses a 3' UTR of the transcript of the SMD target, because the definition of an ALU target site embodies an untranslated region. Additionally, it would have been obvious to one of ordinary skill from the teachings of Maquat and Chiu that binding of Stau1 identifies both transcribed regions as taught by Maquat and UTR regions such as in ALU as taught by Chiu as Stau1 binding sites for SMD targeting and activity. Group II has the special technical feature of identifying a SBS of an SMD target by transiently expressing an ALU element-containing lncRNA linked to a reporter gene and immunoprecipitating the ALU element-containing lncRNA using an anti-reporter protein antibody. This special technical feature is not shared by any of the other Groups. Groups III-V are all directed to a method of screening for an agent that modulates SMD. However, Maquat, as above teaches screening for an agent that modulates SMD (pg 2 ln 1-2; pg 24 ln 22-25; pg 24 ln 30 ? pg 25 ln 18). Group III further requires incubating the agent with a stably transfected cell comprising a reporter gene with a 3' UTR ALU element; a lncRNA containing a corresponding ALU element; and Stau 1, and assaying the amount of mRNA of the reporter gene in the cell, wherein an increase or decrease in the amount of mRNA of the reporter gene relative to the amount of mRNA of the reporter gene in the absence of the agent indicates a substance that modulates SMD activity which is not required by any of the other Groups. Group IV further requires incubating the agent with an SMD target and Stau1 forming a substanceSMD target-Stau1 mixture, and assaying the amount of SMD target-Stau1 complex present in the mixture, wherein an increase or decrease in the amount of SMD target -Stau1 complex relative to the amount of SMD target -Stau 1 complex in the absence of the agent indicates that the substance promotes or inhibits SMD, respectively which is not required by any of the other Groups.

SEE CONTINUATION SHEET.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/24534

Box III, Supplemental Page 1

Group V further requires incubating the agent with an SMD target and Staul forming a substanceSMD target-Staul mixture, and assaying the amount of SMD target present in the mixture, wherein an increase or decrease in the amount of SMD target relative to the amount of SMD target in the absence of the agent indicates that the substance inhibits or promotes SMD, respectively. Group VI requires an agent that modulates SMD. While an agent that modulates SMD is not required, per se, by any of the other Groups (screening of an agent that potentially modulates SMD does not specifically require the agent to be one that actually modulates SMD as per Groups III-V), it is, nonetheless taught by Maquat as above (pg 24 In 21-29). Group VII has the special technical feature of a method of identifying an agent that binds an SBS. This special technical feature is not required by any of the other Groups. Group VIII requires the special technical feature of identifying genes modulated by the down-regulation of an ALU element containing lncRNA. This special technical feature is not required by any of the other Groups. Group IX has the special technical feature of a method of treating a disorder associated with expression of a truncated or erroneous protein in a subject. This special technical feature is not required by any of the other Groups. Therefore the inventions listed as Groups I-IX do not relate to a single general inventive concept under PCT Rule 13.1 because they do not share the same or corresponding special technical features as described. In order for all inventions to be examined, the appropriate examination fees must be paid.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/24534

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

The inventions listed as Groups I+ IV+ do not relate to a single inventive concept under Rule 13.1 because under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Group I claims 1-8 directed to a method of identifying a Staufen 1 (Staul)-binding site (SBS) that mediates Staulmediated mRNA decay (SMD).

Group II claim 9 directed to a method of identifying a SBS of an SMD target.

SEE CONTINUATION SHEET.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-8

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.