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(54) Title: STAUFEN 1 (STAU1) - MEDIATED MRNA DECAY

(57) Abstract: Disclosed are compositions and methods for treating subjects with conditions resulting from Stau 1 -mediated mRNA decay, screening for, and manufacturing those therapeutic agents. Described herein are novel mRNA decay mechanisms that involves mammalian Staufen (Stau)l, the nonsense-mediated mRNA decay (NMD) factor Upfl, and a termination codon. It is shown that Staul binds directly to Upfl and can elicit mRNA decay when tethered downstream of a termination codon. Also disclosed is the new pathway as a means for cells to down-regulate the expression of Staul-binding mRNAs.

STAUFEN 1 (Stau1) – MEDIATED mRNA DECAY

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I. BACKGROUND OF THE INVENTION

2. 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 (Marion et al., 1999; Wickham et al., 1999). The role of Staufen is best characterized in Drosophila, where it functions in the transport and localization of bicoid and oskar mRNAs to, respectively, the anterior and posterior poles of oocytes, and prospero mRNA during asymmetric divisions of embryonic neuroblasts (Broadus et al., 1998; Li et al., 1997; Matsuzaki et al., 1998; Schuldt et al., 1998; Shen et al., 1998; St Johnston, 1995). Drosophila Staufen also functions in the translational derepression of oskar mRNA once the mRNA has been localized to the posterior pole of an oocyte (Ephrussi et al., 1991; Kim-Ha et al., 1995; Kim-Ha et al., 1991; Micklem et al., 2000).

3. In mammals, the Stau1 gene is ubiquitously expressed and generates protein isoforms having apparent molecular weights of 55 and 63 kDa (Kiebler et al., 1999; Marion et al., 1999; Monshausen et al., 2001; Wickham et al., 1999). The 55-kDa isoform associates with 40S and 60S ribosomal subunits and co-localizes with the rough endoplasmic reticulum (Luo et al., 2002; Marion et al., 1999; Wickham et al., 1999). 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 (Kohrmann et al., 1999; Krichevsky and Kosik, 2001; Mallardo et al., 2003; Ohashi et al., 2002; Villace et al., 2004), as well as its encapsidation together with HIV-1 RNA in virus particles (Mouland et al., 2000). Additionally, Stau1 interacts with telomerase RNA, suggesting that it functions during DNA replication, cell division or both, possibly by influencing telomerase RNA processing or RNP assembly or localization (Bachand et al., 2001; Le et al., 2000).

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 treating subjects with conditions that result from or modified by affecting Stau1-mediated mRNA decay and to

screening for and manufacturing those therapeutic agents that modulate Staul mediated mRNA decay.

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.

7. Figure 1 shows that Human Upf1 interacts with human Staufen (Stau)1 in a yeast two-hybrid analyses, in vitro binding assays, and immunopurifications (IPs) of Stau1-HA_3 from Cos cells. Figure 1A shows yeast two-hybrid screening. Human Upf1 as bait interacts with human Stau1 from the pMyr-cDNA library (upper). Negative and positive controls were provided by Stratagene. The region of Stau1 that interacts with Upf1 was mapped to reside within the double-stranded RNA binding domain (dsRBD)4 and tubulin binding domain (TBD) (lower). Figure 1B shows GST pull-down assays. E. coli lysates that expressed GST-Upf1 (+) were mixed with E. coli lysates that did not (-) or did (+) express 6xHis-Stau1, purified using Glutathione Sepharose beads (After) or not (Before), and subjected to Western blotting (WB) using anti-GST antibody (upper) or anti-His antibody (middle) either before or after GST pull-down. Additionally, a fraction of each sample before or after GST-pull-down was analyzed by Coomassie Blue staining to verify the presence of GST-Upf1 and 6xHis-Stau1 (lower, indicated by dots). Figure 1C shows Far-Western analysis. E. coli lysates that did not (-) or did (+) produce either 6xHis-Stau1 or GST-Stau1 were subjected to Far-Western blotting (FW) using FLAG-Upf1 that had been immunopurified from HeLa cells (middle) or GST-Upf1 that had been immunopurified from E. coli (right). Interacting proteins were identified by Western blotting using anti-FLAG or anti-GST antibody, respectively. Expression of 6xHis-Stau1 and GST-Stau1 was also verified by staining each lysate with Coomassie Blue (left). Figure 1D shows IP of Stau1-HA_3. Cos cells were transiently transfected with a plasmid that expressed the 55-kDa

isoform of Staul-HA_3. After cell lysis, RNA and protein were purified from the lysate before and after IP using anti-HA antibody or, to control for the specificity of the IP, rat (r) IgG. RNase A was added to half of each sample prior to IP. SMG7 mRNA was analyzed using RT-PCR to demonstrate that the RNase A digestion was complete (lower). The four left-most lanes represent 2-fold serial dilutions of RNA and demonstrate that the RT-PCR is semi-quantitative. Western blotting was used to detect the specified proteins (upper). The three left-most lanes represent 3-fold serial dilutions of protein before IP and demonstrate that the Western blotting is semi-quantitative. Figure 1E shows IP of cellular Upf3/3X. Lysate from untransfected Cos cells was immunopurified using anti-Upf3/3X antibody or, as a control for nonspecific IP, normal rabbit serum (NRS). Western blotting was used to analyze the specified proteins. Upf3 co-migrated with Ig heavy chains and, thus, could not be analyzed. Figure 1F shows IP of FLAG-Upf1. Lysates of HeLa cells that did (+ pCI-neo-FLAG-UPF1) or did not (- pCI-neo-FLAG-UPF1) stably express FLAG-Upf1 was analyzed either before or after IP using anti-Flag antibody by Western blotting for the specified proteins.

8. Figure 2 shows that down-regulating cellular Staul has no detectable effect on the EJC-dependent NMD of Gl 39Ter or GPx1 46Ter mRNA. HeLa cells were transiently transfected with Staul siRNA, Upf3X siRNA, or a nonspecific Control siRNA. Two days later, cells were re-transfected with pmCMV-Gl and pmCMV-GPx1 test plasmids, either nonsense-free (Norm) or nonsense-containing (Ter), and the phCMV-MUP reference plasmid. After an additional day, protein and RNA were purified. Figure 2A shows the Western blot analysis of the siRNA-mediated down-regulation of Staul or Upf3X, where the level of eIF3b served to control for variations in protein loading. Figure 2B shows the RT-PCR analysis of the level of Gl mRNA (left) or GPx1 mRNA (right), which was normalized to the level of MUP mRNA. Normalized levels of Norm mRNA in the presence of each siRNA were defined as 100%. Levels in three independently performed experiments did not vary by more than 7%.

9. Figure 3 shows that tethering Staul to the FLuc mRNA 3' UTR reduces FLuc mRNA abundance. Figure 3A shows the schematic representations of firefly (F) and renilla (R) luciferase (Luc) expression plasmids pcFLuc-MS2bs, pcFLuc, and pRLuc, where X8 specifies eight tandem repeats of the MS2 coat protein binding site. HeLa cells were co-transfected with the specified pcFLuc-MS2bs reporter plasmid, the pRLuc reference plasmid, as well the specified effector plasmid. Two days after transfection, protein and

RNA were purified. Western blotting using anti-HA antibody demonstrates effector expression (left). RT-PCR demonstrates that tethered Staul reduces FLuc-MS2bs mRNA abundance (right). Numbers below the figure represent the levels of FLuc or FLuc-MS2bs mRNA, which were normalized to the level of RLuc mRNA. Each normalized level of FLuc or FLuc-MS2bs mRNA was then calculated as a percentage of the normalized level of FLuc or FLuc-MS2bs mRNA that was obtained in the presence of pMS2-HA or pcNMS2, which was defined as 100%. RT-PCR results in at least three independently performed experiments did not vary by more than 9%.

10. Figure 4 shows that the abundance of FLuc-MS2bs mRNA is reduced by only specific proteins, such as MS2-HA-Staul or MS2-Upfl, but not MS2-HA-eIF4AIII, myc-Upfl or Staul-HA_3. As in Figure 3, except that the specified effector plasmids were used. Figure 4A shows the western blot analysis of MS2-HA, MS2-HA-Staul and MS2-HA-eIF4AIII expression using anti-HA antibody (left), MS2-Upfl, myc-Upfl and endogenous Upfl expression using anti-Upfl antibody (upper right), and MS2-HA, MS2-HA-Staul and Stau-HA_3 expression using anti-Staul antibody (lower right). Endogenous Staul was detectable with enhanced chemiluminescence. Figure 4B shows RT-PCR analysis of the levels of FLuc-MS2bs and RLuc mRNAs. Numbers below the figure represent the level of FLuc-MS2bs mRNA, which was normalized to the level of RLuc mRNA. Normalized levels were then calculated as a percentage of the normalized level of FLuc-MS2bs mRNA that was obtained in the presence of pMS2-HA, pcNMS2, pCMV-myc, or pCDNA3/RSV (i.e., the vector for hStaul-HA_3 expression), each of which was defined as 100%. Results represent three independently performed experiments and do not vary by more than 10%.

11. Figure 5 shows that siRNA-mediated down-regulation of cellular Upfl but not Upf2 or Upf3X inhibits the reduction in FLuc-MS2bs mRNA abundance that is mediated by tethered Staul. Figure 5A and 5B show that HeLa cells were transiently transfected with the specified siRNA. Two days later, cells were transfected with the pcFLuc-MS2bs reporter plasmid, the pRLuc reference plasmid, and the specified effector plasmid. Alternatively, cells were transfected with a pmCMV-Gl test plasmid (either Norm or Ter) and the phCMV-MUP reference plasmid. After an additional two days, protein and RNA were purified. Figures 5A-5C (left) show that Western blotting was used to quantitate the extent of down-regulation. Figures 5A-5B (middle) shows that RT-PCR was used to quantitate the effects of siRNA on Gl mRNA abundance, as in Figure 2B. Figures 5A-5C (right) show that RT-PCR was used to quantitate the effects of siRNA on mRNA abundance that were

mediated by tethering the specified protein as in Figure 3B. For all RT-PCR results, mRNA levels in at least two independently performed experiments did not differ by more than 10 %.

12. Figure 6 shows evidence that Stau1 reduces mRNA abundance in a way that depends on an upstream termination codon that is located upstream of the Stau1 binding site. HeLa cells were transfected as described in the legend to Figure 3 using the specified test, reference and effector plasmids. Figure 6A shows schematic representations of the pcFLuc-MS2bs, pcFLuc (UAA→CAA)-MS2bs, pcGl-MS2bs, and pcGl(UAA→UAC)-MS2bs test plasmids (the latter two were called pcβ-6bs and pcβUAC-6bs, respectively, in Lykke-Andersen et al., 2000). Figure 6B shows the quantitation of MS2-HA and MS2-HA-Stau1 expression. This figure is like Figure 3, except that pcFLuc(UAA→CAA)-MS2bs was used as the reporter plasmid. Figure 6C is like Figure 3, except that pcGl-MS2bs or pcGl(UAA→UAC)-MS2bs was the reporter plasmid, and phCMV-MUP was the reference plasmid. Results represent two independently performed experiments and do not vary by more than 11%.

13. Figure 7 shows that Stau1 binds Arf1 mRNA. Figure 7A shows the IP of Stau1-HA_3. 293 cells were transiently transfected with a plasmid that expressed Stau1-HA_3 or, to control for nonspecific IP, Stau1-6xHis. Two days later, cells were lysed, a fraction of cell lysates was immunopurified using anti-HA antibody, and Stau1 was identified before and after IP using Western blotting and anti-Stau1 antibody. Asterisks denote the 63-kDa and 55-kDa isoforms of endogenous Stau1. Figure 7B shows the identification of Arf1 mRNA in Stau1-containing RNP. Biotin-labeled cRNA was synthesized from RNA that had been immunopurified using anti-HA antibody from Stau1-HA_3- or Stau1-6xHis-expressing cells or poly(A)$^+$ RNA from untransfected cells. The histogram represents the amount of hybridized Arf1 mRNA that was either immunopurified using anti-HA antibody from Stau1-HA_3-expressing cells (left) or present in untransfected 293-cell poly(A)$^+$ RNA (right), both of which are presented relative to the amount of hybridized Arf1 mRNA that was immunopurified using anti-HA antibody from Stau1-6xHis-expressing cells. A ratio of more than 2.5 is statistically significant. Figure 7C shows RT-PCR of Arf1 mRNA in Stau1-containing RNPs. 293 cells were transiently transfected with a plasmid that expressed either Stau1-HA_3 or, as a control, Stau1-6xHis. As in 10A, except that immunopurified RNA was purified, and Arf1 mRNA was amplified using RT-PCR (two left-most lanes). Alternatively, lysates from untransfected 293 cells were immunopurified

using with anti-Staul antibody (right) or an unrelated ascites fluid for the analysis of Arf1 mRNA.

14. Figure 8 shows that Staul binds the 3' UTR of Arf1 mRNA and reduces its abundance in a mechanism that involves Upf1. Figure 8A shows schematic representations of the Arf1 gene and the pSport-Arf1 and pSport-Arf1Δ(3'UTR) cDNA expression plasmids. Figure 8B is a parallel study to Figure 4A, except that HeLa cells were transiently transfected with the specified siRNA. Two days later, cells were re-transfected with a pSport-Arf1 test plasmid and the reference phCMV-MUP plasmid. After an additional day, protein and RNA were isolated for Western analysis and RT-PCR, respectively. The efficiency of siRNA-mediated down-regulation of gene expression as assessed using Western blotting (upper). The levels of endogenous Arf1 and SMG7 mRNAs (second down) or of Arf1 mRNA that derived from pSport-Arf1 and MUP mRNA (third down) as assessed using RT-PCR. Numbers below the panel represent the level of Arf1 mRNA after normalization to the level of either SMG7 or MUP mRNA, where the normalized level of Arf1 mRNA in the presence of Control siRNA was defined as 100%. Figure 8C is like 8B except that pSport Arf1 or pSport-Arf1Δ(3'UTR) was used as test plasmid. The level of Arf1 mRNA or Arf1Δ(3'UTR) mRNA that derives from pSport-Arf1 and the level of MUP mRNA was assessed using RT-PCR. Figure 8D shows IP of Staul-HA_3. Cos cells were transiently transfected with the Staul-HA_3 expression vector, pSport-Arf1 or pSport-Arf1Δ(3'UTR), and phCMV-MUP. Notably, cells were transfected with only half as much pSport-Arf1Δ(3'UTR) relative to pSport-Arf1 in order to compensate for the difference in the level of product mRNA. Two days later, cells were lysed, and a fraction of lysates was immunopurified using anti-HA antibody or, as a control, rIgG. Protein or RNA before and after IP was analyzed, respectively, using Western blotting and anti-HA antibody (upper), or RT-PCR (lower). For all RT-PCR results, mRNA levels in at least two independently performed experiments did not differ by more than 10 %.

15. Figure 9 shows a comparable increase in the abundance of Arf1 mRNA that derives from pSport-Arf1 is obtained using two different Staul siRNAs and two different Upf1 siRNAs. As in Figure 8B; however, Staul(A) and Upf1(A) siRNAs were analyzed in parallel to, respectively, the Staul and Upf1 siRNAs that were analyzed in the Figure 8B. Figure 9A shows that the western blotting demonstrates down-regulation of Staul (left), and RT-PCR demonstrates that down-regulating Staul increases the level of Arf1 mRNA

abundance (right). Figure 9B is like 9A, except that Upf1 was down-regulated. Results represent two independently performed experiments and do not vary by more than 12%.

16. Figure 10 shows that Stau1 binds within an ~230-nt region of the Arf1 mRNA 3' UTR, and this region reduces the half-life of Arf1 mRNA in a Stau1-mediated mechanism. Figure 10A shows the schematic representations of the various Arf1 mRNAs harboring deletions within the 3' UTR. Numbering is relative to the first nucleotide of endogenous Arf1 mRNA, which is defined as 1. The upper-most construct represents full-length mRNA. Figure 10B shows that the 293 cells were transfected with the Stau1-HA_3 expression vector and the specified pSport-Arf1 test plasmid. One-tenth of each IP was used to determine IP efficiencies using Western blotting (upper). RNA was isolated from the rest of the IP, and the level of Arf1 mRNA from each Sport test plasmid was quantitated by RT-PCR and ethidium bromide staining (lower). The level of Arf1 mRNA from each pSport test plasmid was similarly assessed before IP to control for variations in transfection efficiencies and RNA recovery. Figure 10C shows that the L cells were transfected with mouse (m)Stau1, mUpf1, mUpf2, or Control siRNA. Two days later, cells were re-transfected with the pfos-Arf1-SBS test plasmid (upper left) and the phCMV-MUP reference plasmid in the absence of serum. Serum was added to 15% after an additional 24 hr, and protein was purified from the cytoplasmic fraction for Western blotting (second-from-top, left and right). RNA was purified from the nuclear fraction for RT-PCR analysis at the specified times (second-from-bottom, left and right). For each time point, the level of pfos-Arf1-SBS mRNA was normalized to the level of MUP mRNA. Normalized levels were calculated as a percentage of the normalized level of fos-Arf1-SBS mRNA at 30 min in the presence of each siRNA, which was defined as 100. Normalized levels represent the average of two independently performed experiments and are plotted as a function of time after serum addition (bottom, left and right).

17. Figure 11 shows that inserting the Stau1 binding site (SBS; nts 622-924) of the Arf1 mRNA 3'UTR within FLuc mRNA results in a Stau1-dependent reduction in FLuc mRNA halflife, whereas inserting a different region (No SBS; nts 899-1144) of the Arf1 mRNA 3'UTR does not. Figure 11A shows schematic representations of pcFLuc-SBS and pcFLuc-No SBS test plasmids. Notably, the Arf1 SBS maintains the distance and sequence of the minimized Stau1 binding site (nts 688-919) relative to the normal termination codon. (A, middle and right) As in Figures 5B and C, except that only Stau1 was down-regulated and pcFLuc-SBS or pcFLuc-No SBS were the test plasmids. Western blotting revealed that

Staul siRNA reduced the level of Staul to 11% of normal. Figure 11B was performed as in Figure 10C, except that only mStaul was down-regulated and pfos-FLuc-SBS was the test plasmid. Western blotting revealed that mStaul siRNA reduced the level of mStaul to 29%. Results represent two independently performed experiments, and RT-PCR quantitations did not vary by more than 9% (A) or 29% (B).

18. Figure 12 shows the 3' UTR of PAICS mRNA, which encodes phosphoribosylaminoimidazole carboxylase and phosphoribosylaminoimidazole succinocarboxamide synthetase activities, also binds Staul, and down-regulating Staul increases PAICS mRNA abundance. Figure 12A is like Figure 10B, except that 293 cells were transfected with test plasmid pSport-PAICS or, as a negative control, pCDNA3-RSV-CK2A2, which encodes casein kinase 2 alpha prime polypeptide. Figure 12B is like Figure 8D, except that pSport-PAICS or pSport-PAICSΔ (3'UTR) was the test plasmid. The small amount of MUP mRNA that was detected in the IP represents background since (i) MUP mRNA was never detected in other IPs and (ii) a comparison of the levels of PAICS and MUP mRNAs before and after IP shows a 110–fold enrichment of PAICS mRNA relative to MUP mRNA after IP. Figure 12C is like Figure 8B, except that pSport-PAICS was the test plasmid. Results represent two independently performed experiments and do not vary by more than 2%.

19. Figure 13 shows that down-regulating Staul has no effect on the half-life of fos-Arf1Δ(3'UTR) mRNA. Figure 13 was conduct in the same manner as Figure 11B, except that the test plasmid was pfos-Arf1Δ(3'UTR). Results represent two independently performed experiments, and RT-PCR quantitations did not vary by more than 19%.

20. Figure 14 shows the models for EJC-dependent NMD and SMD of Arf1 mRNA in mammalian cells. Figure 14A shows the recruitment of Upf1 to one of the four EJCs of Arf1 mRNA via Upf2 and Upf3/3X. Within the nucleus, pre-mRNA splicing deposits an exon junction complex (EJC) of proteins that consists of RNPS1, Y14, SRm160, REF/Aly, Mago, UAP56, and eIF4AIII (Chan et al., 2004; Ferraiuolo et al., 2004; Kataoka et al., 2000; Kim et al., 2001; Le Hir et al., 2000b; Lejeune et al., 2002; Luo, et al., 2001; Palacios et al., 2004) and is located ~20-25 nucleotides upstream of each exon-exon junction (Le Hir et al., 2000a). It is unknown if every protein is present within every EJC. The EJC consists of the NMD factor Upf3 or Upf3X, each of which binds Upf2, and Upf2 is thought to subsequently bind Upf1. EJC-dependent NMD occurs if translation terminates prematurely more than ~50-55 nucleotides upstream of any exon-exon junction, which would be ~20-25

nucleotide upstream of the corresponding EJC (only the 3'-most of which is shown). Figure 14B shows the recruitment of Upf1 to the 3' UTR of Arf1 mRNA via Stau1. Stau1, which binds the 3' UTR of Arf1 mRNA, recruits Upf1 independently of an EJC. Data indicate that SMD occurs when translation terminates properly. By analogy to EJC-dependent NMD, the Stau1 binding site would reside more than ~20-25 nts downstream of the normal termination codon in order to elicit SMD. This is consistent with the finding that Stau1 binds at least 67-nt downstream of this codon.

21. Figure 15. Demonstration 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 17. The level of each test transcript was normalized to the level of SMG7 mRNA, which is insensitive to Stau1 siRNA (data not shown) 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.

22. Figure 16. Demonstration that 6 of 6 transcripts that were downregulated in human cells depleted of Stau1 using three independently performed microarray analysis are downregulated using RT-PCR and transcript-specific primers. The experimental procedure was as described in Figure 15.

23. Figure 17 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 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 17A 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 17B shows RT-PCR analysis of the level of endogenous c-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.

24. Figure 18 shows that Stau1 binds within the 3'UTR of c-JUN, SERPINE1 and IL7R mRNAs. Cos cells were transiently transfected with three plasmids: (i) pcFLuc-c-JUN

3'UTR, pcFLuc-SERPINE1 3'UTR and pcFLuc-IL7R 3'UTR test plasmids, which encode FLuc mRNA in which all of the FLuc 3'UTR has been replaced by, respectively, part of the c-JUN 3'UTR or all of the SERPINE1 or IL7R 3'UTR; (ii) the Stau1-HA_3 expression vector; (iii) the pcFLuc-SBS test plasmid, which encodes FLuc mRNA in which the 3'UTR has been replaced by the 231-nucleotide Stau1 binding site (SBS) of Arf1 mRNA (Kim et al., 2005) and serves as a positive control for Stau1-HA_3 binding; and (iv) phCMV-MUP, which encodes MUP mRNA that serves as a negative control for Stau1-HA_3 binding. Two days later, cells were lysed, and a fraction of each lysate was immunopurified using anti-HA or, as a control for immunopurification (IP) specificity, rat (r)IgG. Figure 18A shows schematic representations of pcFLuc-c-JUN, pcFLuc-SERPINE1, pcFLuc-IL7R and pcFLuc-SBS test plasmids. Figure 18B shows Western blotting using anti(α)-HA or anti-Calnexin demonstrated that Stau1-HA_3 but, as expected, not Calnexin was immunopurified. Figure 18C shows RT-PCR analysis demonstrates that c-JUN, SERPINE1 and IL7R 3'UTRs, like the Arf1 SBS, bind Stau1-HA_3, whereas MUP mRNA does not. Results are representative of three independently performed experiments.

25. Figure 19 shows that depleting cells of Stau1 or Upf1 increases the half-life of FLuc mRNA when the 3'UTR consists of the Stau1 binding site of the c-JUN, SERPINE1 or IL7R 3'UTR. L cells were transfected with mouse (m)Stau1, mUpf1 or Control siRNA. Two days later, cells were re-transfected with the pfos-FLuc-Arf1 SBS, pfos-FLuc-c-JUN 3'UTR, pfos-FLuc-SERPINE1 3'UTR or pfos-FLuc-IL7R 3'UTR test plasmid and the phCMV-MUP reference plasmid in the absence of serum. Serum was added to 15% after an additional 24 hours. Protein was immediately purified from the cytoplasmic fraction (at 0 minutes) for Western blotting. RNA was purified from the nuclear fraction for RT-PCR analysis at the specified times. Figure 19A shows schematic representations of each test plasmid. Figure 19B shows Western blotting of mStau1 or mUpf1, where the level of β-actin serves to control for variations in protein loading. mStau1 was depleted to 35% its normal level, and mUpf1 was depleted to 30% of its normal level. Figure 19C shows RT-PCR analysis of the SMD candidate mRNAs. For each time point, the level of each mRNA transcribed from the fos promoter is normalized to the level of MUP mRNA. Normalized levels are calculated as a percentage of the normalized level of each mRNA transcribed at 30 minutes in the presence of each siRNA, which is defined as 100 (left). These levels are plotted as a function of time after serum addition (right). Error bars specify the extent of variation among two or three independently performed experiments.

26. Figure 20 shows that GAP43 mRNA is an SMD target. Figure 20A shows RT-PCR demonstrating that depleting cells of Stau1 or Upf1 increases the cellular abundance of GAP43 mRNA. RNA derived from samples used in Figure 1. Figure 20B shows IP revealing that Stau1 binds to the 3' UTR of GAP43 mRNA and, as a positive control Arf1 SBS. Cos cells were transfected with three plasmids: (i) pcFLuc-GAP43 3' UTR (left), (ii) the Stau1-HA_3 expression vector; (iii) pcFLuc-SBS (left), and (iv) phCMV-MUP and analyzed as described in the legend to Figure 2 except that FLuc-GAP43 3' UTR and FLuc-Arf1 SBS mRNAs were analyzed instead of the other FLuc mRNA derivatives. Figure 20C shows Western blot (upper right) and RT-PCR analysis (lower left) demonstrating that depleting cells of Stau1 or Upf1 increases the half-life of FLuc mRNA when the 3'UTR consists of the Stau1 binding site of GAP43 mRNA. L cells were transfected with mStau1, mUpf1 or Control siRNA. Two days later, cells were re-transfected with the pfos-FLuc-GAP43 3' UTR test plasmid and the phCMV-MUP reference plasmid in the absence of serum. Subsequent treatment and analyses were as described in the legend to Figure 19 except that fos-FLuc-GAP43 3' UTR mRNA was analyzed instead of the other fos-FLuc mRNA derivatives. Results are representative of two independently performed experiments.

IV. DETAILED DESCRIPTION

27. 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.

28. Before the present compounds, compositions, articles, devices, and/or methods 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.

29. 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.

30. 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.

31. 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:

32. "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.

33. "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.

34. "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.

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

36. 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.

37. Staul is involved in mRNA decay. Also, Staul-mediated mRNA decay involves the nonsense-mediated mRNA decay (NMD) factor Upf1. In mammalian cells, the 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 (reviewed in Maquat and Carmichael, 2001). 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 (reviewed in Frischmeyer and Dietz, 1999; Hentze and Kulozik, 1999; Li and Wilkinson, 1998; Maquat, 2004a; Maquat, 2004b; Wilusz et al., 2001). By so doing, NMD precludes the synthesis of the encoded truncated proteins, which can function in deleterious ways (see, e.g., Inoue et al., 2004). 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 (Maquat, 2004b).

38. 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 (Kataoka et al., 2000; Kim et al., 2001; Le Hir et al., 2000a; Le Hir et al., 2000b; Lykke-Andersen et al., 2001). This EJC includes NMD factors Upf3 (also called Upf3a) or Upf3X (also called Upf3b), Upf2 and, presumably, Upf1 (Gehring et al., 2003; Kim et al., 2001; Lejeune et al., 2002; Lykke-Andersen et al., 2000; Lykke-Andersen et al., 2001). Upf3 and Upf3X appear to play a comparable role in NMD (Kim et al., 2001; Lykke-Andersen et al., 2001), although different isoforms of Upf3 can form distinct protein complexes (Ohashi et al., 2002). Other constituents of the EJC include Y14, RNPS1, SRm160, REF/Aly, UAP56, Mago, Pinin, and eIF4AIII (Chan et al., 2004; Ferraiuolo et al., 2004; Kataoka et al., 2000; Kim et al., 2001; Le Hir et al., 2001; Le Hir et al., 2000b; Luo, et al., 2001; Palacios et al., 2004; Shibuya et al., 2004; Tarn et al., 2003). 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 (Chiu et al., 2004; Ishigaki et al., 2001; Lejeune et al., 2002; Lejeune et al., 2004).

39. Mammalian Staul binds the NMD factor Upf1 and the 3' untranslated region (UTR) of mRNA that encodes ADP-ribosylation factor (Arf)1. As a consequence, Staul mediates Arf1 mRNA decay in a mechanism that differs from NMD by occurring independently of splicing or Upf2 and Upf3X. Analogously to the Staul-mediated mRNA decay (SMD) of Arf1 mRNA, artificially tethering Staul 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, Staul plays no

detectable role in the EJC-dependent NMD of either β-globin or glutathione peroxidase 1 mRNA.

40. The invention described herein in one aspect relates to methods of treating a disorder in a subject comprising administering to the subject a substance, wherein the substance modulates Stau1-mediated mRNA decay (SMD). 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 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.

41. 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, 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, lysomal enzymes disorder, steroid metabolism disorder, purine metabolism disorder, pyrimidine metabolism disorder, metal metabolism disorder, porphyrin metabolism disorder, and heme metabolism disorder.

42. 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.

43. 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.

44. 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.

45. The disclosed compositions can be used to treat any disease where uncontrolled 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, plasmacytoma, 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.

46. Disclosed and herein contemplated are methods wherein the SMD occurs in or 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.

47. 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 embodiment the subject is a mammal. It is specifically contemplated that mammal can include but is not limited to human, monkey, mouse, dog, pig, rabbit, and cow.

48. 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 subject with a condition resulting from a mutation that generates a nonsense codon (including but not limited to a nonsense mutatation) 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 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 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 alternative termination codon, providing another means by which gene expression could be regulated.

49. 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 to a reduction in an activity. A substance is also understood to decrease the genetic output of a gene when the genetic output of the gene product with the substance is less relative to the output of the gene product without the substance. 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 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.

50. Also disclosed are methods wherein the modulation is an increase in Staul-mediated mRNA decay. An "increase" can refer to any change that results in a larger amount of a Staul 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 contemplated that an increase in SMD would result in a subsequent decrease in the amount of gene products with early or alternative termination sites.

51. It is understood and herein contemplated that Staul can bind mRNA at AU rich elements (ARE). For example, it is contemplated that Staul binds mRNA at Type III AREs. The binding of Staul to AREs can affect the level of gene expression for the mRNA transcript to which it binds. It is also understood and herein contemplated that the binding of Staul to AREs can have a stabilizing effect. Therefore, herein disclosed is the ability of Staul to bind stabilizing or destabilizing elements. Thus, herein contemplated are methods wherein the modulation of SMD results in the stabilization of mRNA. Also disclosed are methods wherein the modulation of SMD results in the destabilization of mRNA.

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

53. It is understood and herein contemplated that the disclosed methods can be used to modulate the level of mRNA or protein expression. It is also understood and herein contemplated that Staul can increase or decrease the abundance of an mRNA independently of SMD. Likewise, it is understood and herein contemplated that not all SMD activity occurs through the involvement of Staul 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 Staul. The ability to modulate the abundance of target genes can be achieved in conjunction with or independently of Upf1. It is understood that such effect can be either direct or indirect. By "direct effect" is meant that Staul acts on the targeted mRNA itself and by "indirect effect" is meant that Staul acts by an intermediary, including for example, a nucleic acid. Thus, Staul down-regulation can result

either directly or indirectly in the down reulation of target mRNA levels. It is understood that whether the effect is direct or indirect will depend 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 Staul comprising a) incubating a substance that down-regulates SMD with a stably transfected cell comprising a reporter gene with a nonsense-mutation and Staul, and b) assaying the amount of mRNA present for a gene of a microarray, wherein a increase or decrease in the amount of mRNA relative to the amount of mRNA in the absence of the substance indicates a gene that is modulated by Staul activity. It is understood that these methods can be used to identify genes that are up-regulated or down regulated by the down-regulation of Staul as well as identify those genes whose abundance increases or decreases with the down-regulation of Staul. It is understood and herein contemplated that one example of a substance that can down regulate SMD is small interfering RNA (siRNA) to a componenet of SMD such as Staul or Upfl. Thus, for example, disclosed herein are methods of identifying genes modulated by the down-regulation of Staul comprising a) transfecting a small interfering RNA (siRNA) that down-regulates SMD into a cell comprising Staul 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 Staul activity. In these methods, the siRNA can be for example, Staul siRNA. The methods can also be used to identify genes that are stabilized or destabilized by the down-regulation of Staul.

54. Although Staul can modulate mRNA levels independently of Upfl and SMD, dependent modulation can also occur. Thus, disclosed herein are methods of identifying genes modulated by the down-regulation of Upfl comprising a) transfecting a small interfering RNA (siRNA) that down-regulates SMD into a cell comprising Upfl 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 Upfl 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, 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.

55. 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. 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 preotein expression or mRNA levels known to those of skill in the art.

56. Also disclosed are methods of modulating the level of an mRNA comprising administering to a subject an effective amount of a substance that modulates Stau1, wherein 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 a substance, wherein the substance modulates Stau1 wherein the modulation of Stau1 modulates that level of mRNA abundance of another gene.

57. The disclosed methods make use of substances administered to a subject to achieve a desired effect. It is understood and herein contemplated that "substance" can refer to any agent, compound, functional nucleic acid, siRNA, peptide, protein, antibody, or small molecule. Thus, for example, one embodiment of the disclosed methods is a method of treating a subject with a substance, wherein the substance is Stau1 or a complex comprising Stau1 and Upf1. It is understood that by "SMD complex" is any combination of one or 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.

58. Also disclosed is a substance that modulates SMD, wherein the substance is an antibody or fragment thereof that modulates SMD. For example, the antibody can be an antibody that binds Upf1 or Stau1 and affects SMD activity. Thus, specifically disclosed is antibody, wherein the antibody binds Stau1. Also disclosed is an antibody, wherein the antibody binds Upf1.

59. A substance that modulates SMD can also be a nucleic acid. Therefore specifically disclosed and herein contemplated is a substance, wherein the substance 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.

60. Thus specifically contemplated and disclosed herein is a substance that 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.

61. Thus, specifically disclosed are methods of treating a Stau1-mediated mRNA decay related disorder in a subject comprising administering to the subject Stau1 or a complex comprising Stau1 and Upf1. Also disclosed are methods, wherein the substance binds Upf1 or Stau1. As used herein, "binds" or "interacts" means to affect a substance either directly or indirectly through cooperative function, competitive inhibition, non-competitive inhibition, binding, or contacting the substance, 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.

62. 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 contacting with Upf1 a system, wherein the system comprises the components for SMD. Also disclosed are methods of facilitating Stau1-mediated mRNA decay comprising contacting with Stau1 and Upf1, a system comprising the components for SMD. The combination of Stau1 and Upf1 ccan be simultaneous or sequential.

63. As described above, the compositions can also be administered *in vivo* in a pharmaceutically acceptable carrier. By "pharmaceutically acceptable" is meant a material 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 side effects in the subject, as would be well known to one of skill in the art.

64. 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 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 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 can be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein.

65. 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 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.

66. 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 (Senter, et al., *Bioconjugate Chem.*, 2:447-451, (1991); Bagshawe, K.D., *Br. J. Cancer*, 60:275-281, (1989); Bagshawe, et al., *Br. J. Cancer*, 58:700-703, (1988); Senter, et al., *Bioconjugate Chem.*, 4:3-9, (1993); Battelli, et al., *Cancer Immunol. Immunother.*, 35:421-425, (1992); Pietersz and McKenzie, *Immunolog. Reviews*, 129:57-80, (1992); and Roffler, et al., *Biochem. Pharmacol*, 42:2062-2065, (1991)). 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 (Hughes et al., *Cancer Research*, 49:6214-6220, (1989); and Litzinger and Huang, *Biochimica et Biophysica Acta*, 1104:179-187, (1992)). 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)).

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

68. 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.

69. 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.

70. 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 transdermally.

71. 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 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, anti-oxidants, chelating agents, and inert gases and the like.

72. 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.

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

74. 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 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.

75. The dosage ranges for the administration of the compositions are those large 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.

76. 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, the cell cycle. There use as exogenous DNA delivery devices can be expanded for nearly any reason desired by those of skill in the art.

77. 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 significantly (as determined by the manufacturers specifications of the array) increased or decreased expression are comodulators of SMD.

78. 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 of the peptide sequences disclosed herein.

79. 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.

80. A substance 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 a substance that modulates Stau1-mediated mRNA decay (SMD) comprising incubating the substance to be screened with a Stau1 mRNA decay complex and assaying for a change in SMD. An increase or decrease in SMD activity indicates a modulating substance. 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 Upf1 and Stau1. Also disclosed are screening methods wherein the complex comprises one of Upf1 and Stau1.

81. Disclosed is a method of screening for a substance that modulates Stau1-mediated mRNA decay (SMD) comprising incubating the substance 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 substance indicates a substance that modulates SMD activity. Therefore, specifically disclosed are methods of screening for a substance that inhibits Stau1-mediated mRNA decay (SMD) comprising incubating the substance with Upf1 and Stau1 forming a substance-Upf1-Stau1 mixture, and assaying the amount of Upf1-Stau1complex present in the mixture, a decrease in the amount of Upf1-Stau1complex relative to the amount of Upf1-Stau1complex in the absence of the substance indicating the substance inhibits SMD. Also disclosed are method of screening for a substance that promotes Stau1-mediated mRNA decay (SMD) comprising incubating the substance with Upf1 and Stau1 forming a substance- Upf1-Stau1 mixture, and assaying the amount of Upf1-Stau1 complex present in the mixture, wherein a increase in the amount of Upf1-Stau1 complex relative to the amount of Upf1-Stau1 complex in the absence of the substance indicates that the substance promotes SMD. It is understood that many fragments or minor variants of the disclosed Stau1 and Upf1 proteins 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. Also disclosed are screening methods wherein the Upf1 has at least 80%, 85%, 90%, or 95% identity to the sequence set forth in SEQ ID NO: 4, or an SMD-active fragment thereof.

82. Disclosed herein are methods of screening for a substance that modulates Stau1-mediated mRNA decay (SMD) comprising, administering a substance to a system, wherein the system comprises the components for SMD activity, and assaying the effect of the substance 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 substance indicates the substance is a modulator. Thus, specifically contemplated and herein disclosed are methods of modulating Stau1-mediated mRNA decay (SMD) activity comprising administering a substance, wherein the substance is identified by the disclosed screening methods. Also disclosed are methods of making a substance that modulates Stau1-mediated mRNA decay (SMD) activity comprising admixing a substance identified by the disclosed screening methods with a pharmaceutically acceptable carrier.

83. The agents that can be used to modulate SMD can function by inhibiting the binding of Stau1 to its binding site. For example, a substance 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 the Stau1 binding site comprises SEQ ID NO: 56.

84. 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, 3, 4, and 56 or portions thereof, are used as the target in a combinatorial or screening protocol.

85. 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 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.

86. 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 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 sequences and subjects that complex mixture, for example, approximately 10^{15} individual sequences in 100 μg 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. 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.

87. 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. (See for example, United States Patent No. 6,031,071; 5,824,520; 5,596,079; and 5,565,332 which are herein incorporated by reference at least for their material related to phage display and methods relate to combinatorial chemistry)

88. 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, 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 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 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 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 and are preferably performed as in Roberts and Szostak (Roberts R.W. and Szostak J.W. Proc. Natl. Acad. Sci. USA, 94(23)12997-302 (1997)).

89. 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. 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 (Fields and Song, *Nature* 340:245-6 (1989)). 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 intracellular environment. The method utilizes a library of peptide molecules that attached to an acidic activation domain.

90. 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 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.

91. Techniques for making combinatorial libraries and screening combinatorial libraries to isolate molecules which bind a desired target are well known to those of skill in the art. Representative techniques and methods can be found in but are not limited to United States patents 5,084,824, 5,288,514, 5,449,754, 5,506,337, 5,539,083, 5,545,568, 5,556,762, 5,565,324, 5,565,332, 5,573,905, 5,618,825, 5,619,680, 5,627,210, 5,646,285, 5,663,046, 5,670,326, 5,677,195, 5,683,899, 5,688,696, 5,688,997, 5,698,685, 5,712,146, 5,721,099, 5,723,598, 5,741,713, 5,792,431, 5,807,683, 5,807,754, 5,821,130, 5,831,014, 5,834,195, 5,834,318, 5,834,588, 5,840,500, 5,847,150, 5,856,107, 5,856,496, 5,859,190, 5,864,010, 5,874,443, 5,877,214, 5,880,972, 5,886,126, 5,886,127, 5,891,737, 5,916,899, 5,919,955, 5,925,527, 5,939,268, 5,942,387, 5,945,070, 5,948,696, 5,958,702, 5,958,792, 5,962,337, 5,965,719, 5,972,719, 5,976,894, 5,980,704, 5,985,356, 5,999,086, 6,001,579, 6,004,617, 6,008,321, 6,017,768, 6,025,371, 6,030,917, 6,040,193, 6,045,671, 6,045,755, 6,060,596, and 6,061,636.

92. Combinatorial libraries can be made from a wide array of molecules using a number of different synthetic techniques. For example, libraries containing fused 2,4-pyrimidinediones (United States patent 6,025,371) dihydrobenzopyrans (United States Patent 6,017,768and 5,821,130), amide alcohols (United States Patent 5,976,894), hydroxy-

amino acid amides (United States Patent 5,972,719) carbohydrates (United States patent 5,965,719), 1,4-benzodiazepin-2,5-diones (United States patent 5,962,337), cyclics (United States patent 5,958,792), biaryl amino acid amides (United States patent 5,948,696), thiophenes (United States patent 5,942,387), tricyclic Tetrahydroquinolines (United States patent 5,925,527), benzofurans (United States patent 5,919,955), isoquinolines (United States patent 5,916,899), hydantoin and thiohydantoin (United States patent 5,859,190), indoles (United States patent 5,856,496), imidazol-pyrido-indole and imidazol-pyrido-benzothiophenes (United States patent 5,856,107) substituted 2-methylene-2, 3-dihydrothiazoles (United States patent 5,847,150), quinolines (United States patent 5,840,500), PNA (United States patent 5,831,014), containing tags (United States patent 5,721,099), polyketides (United States patent 5,712,146), morpholino-subunits (United States patent 5,698,685 and 5,506,337), sulfamides (United States patent 5,618,825), and benzodiazepines (United States patent 5,288,514).

93. As used herein combinatorial methods and libraries included traditional screening methods and libraries as well as methods and libraries used in interative processes.

94. 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.

95. 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, Staul and Upfl, are also disclosed. Thus, the products produced using the molecular modeling approaches that involve the disclosed compositions, such as, Staul and Upfl, are also considered herein disclosed.

96. 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.

97. 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.

98. A number of articles review computer modeling of drugs interactive with specific proteins, such as Rotivinen, et al., 1988 *Acta Pharmaceutica Fennica* 97, 159-166; Ripka, *New Scientist* 54-57 (June 16, 1988); McKinaly and Rossmann, 1989 *Annu. Rev. Pharmacol. Toxiciol.* 29, 111-122; Perry and Davies, *QSAR: Quantitative Structure-Activity Relationships in Drug Design* pp. 189-193 (Alan R. Liss, Inc. 1989); Lewis and Dean, 1989 *Proc. R. Soc. Lond.* 236, 125-140 and 141-162; and, with respect to a model enzyme for nucleic acid components, Askew, et al., 1989 *J. Am. Chem. Soc.* 111, 1082-1090. Other computer programs that screen and graphically depict chemicals are available from companies such as BioDesign, Inc., Pasadena, CA., Allelix, Inc, Mississauga, Ontario, Canada, and Hypercube, Inc., Cambridge, Ontario. Although these are primarily designed for application to drugs specific to particular proteins, they can be adapted to design of molecules specifically interacting with specific regions of DNA or RNA, once that region is identified.

99. 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.

100. 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.

101. 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 (See, U.S. Pat. No. 4,816,567 and Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)).

102. 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.

103. The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Pat. No. 4,816,567 (Cabilly et al.). 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, e.g., as described in U.S. Patent No. 5,804,440 and U.S. Patent No. 6,096,441.

104. *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. Examples of papain digestion are described in WO 94/29348 and U.S. Pat. No. 4,342,566. 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.

105. 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 fragment. (Zoller, M.J. *Curr. Opin. Biotechnol.* 3:348-354, 1992).

106. 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 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.

107. The human antibodies of the invention can be prepared using any technique. Examples of techniques for human monoclonal antibody production include those described by Cole et al. (*Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77, 1985) and by Boerner et al. (*J. Immunol.*, 147(1):86-95, 1991). Human antibodies of the invention (and fragments thereof) can also be produced using phage display libraries (Hoogenboom et al., *J. Mol. Biol.*, 227:381, 1991; Marks et al., *J. Mol. Biol.*, 222:581, 1991).

108. 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 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. Antibodies having the desired activity are selected using Env-CD4-co-receptor complexes as described herein.

109. 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 portion of an antigen binding site from a non-human (donor) antibody integrated into the framework of a human (recipient) antibody.

110. 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. 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 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 (Jones et al., *Nature*, 321:522-525 (1986), Reichmann et al., *Nature*, 332:323-327 (1988), and Presta, *Curr. Opin. Struct. Biol.*, 2:593-596 (1992)).

111. 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 (Jones et al., *Nature*, 321:522-525 (1986), Riechmann et al., *Nature*, 332:323-327 (1988), Verhoeyen et al., *Science*, 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Methods that can be used to produce humanized antibodies are also described in U.S. Patent No. 4,816,567, U.S. Patent No. 5,565,332, U.S. Patent No. 5,721,367, U.S. Patent No. 5,837,243, U.S. Patent No. 5, 939,598, U.S. Patent No. 6,130,364, and U.S. Patent No. 6,180,377.

112. 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 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. Guidance in selecting appropriate doses for antibodies is found in the literature on therapeutic uses of antibodies, e.g., Handbook of Monoclonal Antibodies, Ferrone et al., eds., Noges Publications, Park Ridge, N.J., (1985) ch. 22 and pp. 303-357; Smith et al., Antibodies in Human Diagnosis and Therapy, Haber et al., eds., Raven Press, New York (1977) pp. 365-389. 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.

113. 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.

114. 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), 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).

115. As one example, vector delivery can be via a viral system, such as a retroviral vector system which can package a recombinant retroviral genome (see e.g., Pastan et al., *Proc. Natl. Acad. Sci. U.S.A.* 85:4486, 1988; Miller et al., *Mol. Cell. Biol.* 6:2895, 1986). 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 (Mitani et al., *Hum. Gene Ther.* 5:941-948, 1994), adeno-associated viral (AAV) vectors (Goodman et al., *Blood* 84:1492-1500, 1994), lentiviral vectors (Naidini et al., *Science* 272:263-267, 1996), pseudotyped retroviral vectors (Agrawal et al., *Exper. Hematol.* 24:738-747, 1996). Physical transduction techniques can also be used, such as liposome delivery and receptor-mediated and other endocytosis mechanisms (see, for example, Schwartzenberger et al., *Blood* 87:472-478, 1996). This invention can be used in conjunction with any of these or other commonly used gene transfer methods.

116. 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 (Crystal, *Hum. Gene Ther.* 8:985-1001, 1997; Alvarez and Curiel, *Hum. Gene Ther.* 8:597-613, 1997). 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.

117. 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, see, e.g., *Remington: The Science and Practice of Pharmacy* (19th ed.) ed. A.R. Gennaro, Mack Publishing Company, Easton, PA 1995.

118. 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 Staul or Upfl is disclosed and discussed and a number of modifications that can be made to a number of molecules including the Staul or Upfl are discussed, specifically contemplated is each and every combination and permutation of Staul and Upfl 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.

119. 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, Staul 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 Upfl or a

nucleic acid encoding Upf1 has at least 80%, 85%, 90%, or 95% identity to the sequence set forth in SEQ ID NO: 3 or 4, or fragment thereof.

120. 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.

121. 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.

122. 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.

123. 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 advantagous that the antisense molecule be made up of nucleotide analogs that reduce the degradation of the antisense molecule in the cellular environment.

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

125. 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 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 (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. 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, Crooke, 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. 5-methylcytosine can increase the stability of duplex formation. Often time base modifications can be combined with for example a sugar modifcation, such as 2'-O-methoxyethyl, to achieve unique properties such as increased duplex stability. There are numerous United States patents such as 4,845,205; 5,130,302; 5,134,066; 5,175,273;

5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; and 5,681,941, which detail and describe a range of base modifications. Each of these patents is herein incorporated by reference.

126. Nucleotide analogs can also include modifications of the sugar moiety. Modifications to the sugar moiety would include natural modifications of the ribose and 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 C_1 to C_{10}, alkyl or C_2 to C_{10} alkenyl and alkynyl. 2' sugar modiifcations 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 $-O(CH_2)_nON[(CH_2)_n CH_3)]_2$, where n and m are from 1 to about 10.

127. Other modifications at the 2' position include but are not limted 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. There are numerous United States patents that teach the preparation of such modified sugar structures such as 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, each of which is herein incorporated by reference in its entirety.

128. 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. Numerous United States patents teach how to make and use nucleotides containing modified phosphates and include but are not limited to, 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050, each of which is herein incorporated by reference.

129. 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.

130. 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.

131. 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 CH_2 component parts. Numerous United States patents disclose how to make and use these types of phosphate replacements

and include but are not limited to 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439, each of which is herein incorporated by reference.

132. 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. Let., 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. Let., 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. Numerous United States patents teach the preparation of such conjugates and include, but are not limited to U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941, each of which is herein incorporated by reference.

133. 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.

134. 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 adjust the compositions and methods relating to a particular sequence to other related sequences (i.e. sequences of Upf1). Primers and/or probes can be designed for any Stau1 or Upf1 sequence given the information disclosed herein and known in the art.

135. Functional nucleic acids are nucleic acid molecules that have a specific 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 affectors, inhibitors, modulators, and stimulators of a specific activity possessed by a target molecule, or the functional nucleic acid molecules can possess a de novo activity independent of any other molecules.

136. 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 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 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 moelcule 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}.

A representative sample of methods and techniques which aid in the design and use of antisense molecules can be found in the following non-limiting list of United States patents: 5,135,917, 5,294,533, 5,627,158, 5,641,754, 5,691,317, 5,780,607, 5,786,138, 5,849,903, 5,856,103, 5,919,772, 5,955,590, 5,990,088, 5,994,320, 5,998,602, 6,005,095, 6,007,995, 6,013,522, 6,017,898, 6,018,042, 6,025,198, 6,033,910, 6,040,296, 6,046,004, 6,046,319, and 6,057,437.

137. 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 (United States patent 5,631,146) and theophiline (United States patent 5,580,737), as well as large molecules, such as reverse transcriptase (United States patent 5,786,462) and thrombin (United States patent 5,543,293). Aptamers can bind very tightly with k_ds 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. Representative examples of how to make and use aptamers to bind a variety of different target molecules can be found in the following non-limiting list of United States patents: 5,476,766, 5,503,978, 5,631,146, 5,731,424 , 5,780,228, 5,792,613,

5,795,721, 5,846,713, 5,858,660 , 5,861,254, 5,864,026, 5,869,641, 5,958,691, 6,001,988, 6,011,020, 6,013,443, 6,020,130, 6,028,186, 6,030,776, and 6,051,698.

138. It is understood herein that "siRNA" referes 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 Staul and Upfl. 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 (for example, WO 02/44321; Gitlin et al., Nature 418:430-4, 2002; Caplen et al., Proc. Natl. Acad. Sci. 98:9742-9747, 2001; and Elbashir et al., Nature 411:494-8, 2001). In certain examples, siRNAs are directed against certain target genes to down regulate gene expression. For example, Staul or Upfl expression can be down regulated by specifically targeting the siRNA to Staul or Upfl.

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

140. 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
isolelucine	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

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

141. 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.

142. 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.

143. 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.

144. 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 asparyl 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]), acetylation of the N-terminal amine and, in some instances, amidation of the C-terminal carboxyl.

145. 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 particular sequence of Stau1 and SEQ ID NO: 4 sets forth a particular sequence of a Upf1 protein. 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.

146. 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.

147. 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.

148. 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% homology to a particular sequence wherein the variants are conservative mutations.

149. 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 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 or Upf1 are also disclosed including for example SEQ ID NO:1 and SEQ ID NO:3 which set forth two of the degenerate nucleic acid sequences that encode the particular polypeptide set forth in SEQ ID NO:2 and 4, respectively. 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.

150. 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 modifed 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.

151. 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 (Ram et al. *Cancer Res.* 53:83-88, (1993)).

152. 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.

153. 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.

154. A retrovirus is an animal virus belonging to the virus family of Retroviridae, including any types, subfamilies, genus, or tropisms. Retroviral vectors, in general, are described by Verma, I.M., Retroviral vectors for gene transfer. In Microbiology-1985, American Society for Microbiology, pp. 229-232, Washington, (1985), which is incorporated by reference herein. Examples of methods for using retroviral vectors for gene therapy are described in U.S. Patent Nos. 4,868,116 and 4,980,286; PCT applications WO 90/02806 and WO 89/07136; and Mulligan, (Science 260:926-932 (1993)); the teachings of which are incorporated herein by reference.

155. 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.

156. 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.

157. The construction of replication-defective adenoviruses has been described (Berkner et al., *J. Virology* 61:1213-1220 (1987); Massie et al., *Mol. Cell. Biol.* 6:2872-2883 (1986); Haj-Ahmad et al., *J. Virology* 57:267-274 (1986); Davidson et al., *J. Virology* 61:1226-1239 (1987); Zhang "Generation and identification of recombinant adenovirus by liposome-mediated transfection and PCR analysis" BioTechniques 15:868-872 (1993)). 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 (Morsy, *J. Clin. Invest.* 92:1580-1586 (1993); Kirshenbaum, *J. Clin. Invest.* 92:381-387 (1993); Roessler, *J. Clin. Invest.* 92:1085-1092 (1993); Moullier, *Nature Genetics* 4:154-159 (1993); La Salle, *Science* 259:988-990 (1993); Gomez-Foix, *J. Biol. Chem.* 267:25129-25134 (1992); Rich, *Human Gene Therapy* 4:461-476 (1993); Zabner, *Nature Genetics* 6:75-83 (1994); Guzman, *Circulation Research* 73:1201-1207 (1993); Bout, *Human Gene Therapy* 5:3-10 (1994); Zabner, *Cell* 75:207-216 (1993); Caillaud, *Eur. J. Neuroscience* 5:1287-1291 (1993); and Ragot, *J. Gen. Virology* 74:501-507 (1993)). 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 (Chardonnet and Dales, Virology 40:462-477 (1970); Brown and Burlingham, J. Virology 12:386-396 (1973); Svensson and Persson, J. Virology 55:442-449 (1985); Seth, et al., J. Virol. 51:650-655 (1984); Seth, et

al., Mol. Cell. Biol. 4:1528-1533 (1984); Varga et al., J. Virology 65:6061-6070 (1991); Wickham et al., Cell 73:309-319 (1993)).

158. A viral vector can be one based on an adenovirus which has had the E1 gene removed and these virons 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.

159. 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 the herpes simplex virus thymidine kinase gene, HSV-tk, and/or a marker gene, such as the gene encoding the green fluorescent protein, GFP.

160. 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 refers to any nucleotide sequence or gene which is not native to the AAV or B19 parvovirus.

161. 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 expression. United states Patent No. 6,261,834 is herein incorproated by reference for material related to the AAV vector.

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

163. 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 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.

164. Molecular genetic experiments with large human herpesviruses have 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. 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.

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

166. 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 example in vivo or in vitro.

167. 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 administered to the blood afferent to a target organ or inhaled into the respiratory tract to target cells of the respiratory tract. Regarding liposomes, see, e.g., Brigham et al. *Am. J. Resp. Cell. Mol. Biol.* 1:95-100 (1989); Felgner et al. *Proc. Natl. Acad. Sci USA* 84:7413-7417 (1987); U.S. Pat. No.4,897,355. 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.

168. 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 (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).

169. 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 (Senter, et al., *Bioconjugate Chem.*, 2:447-451, (1991); Bagshawe, K.D., *Br. J. Cancer*, 60:275-281, (1989); Bagshawe, et al., *Br. J. Cancer*, 58:700-703, (1988); Senter, et al., *Bioconjugate Chem.*, 4:3-9, (1993); Battelli, et al., *Cancer Immunol. Immunother.*, 35:421-425, (1992); Pietersz and McKenzie, *Immunolog. Reviews*, 129:57-80, (1992); and Roffler, et al., *Biochem. Pharmacol*, 42:2062-2065, (1991)). These techniques can be used for a variety of other speciifc 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 following references are examples of the use of this technology to target specific proteins to tumor tissue (Hughes et al., *Cancer Research*, 49:6214-6220, (1989); and Litzinger and Huang, *Biochimica et Biophysica Acta*, 1104:179-187, (1992)). 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)).

170. 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 intergration systems can also be incorporated into nucleic acids which are to be delivered using a non-nucleic acid based system of deliver, such as a liposome, so that the nucleic acid contained in the delivery system can be come integrated into the host genome.

171. 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 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.

172. As described above, the compositions can be administered in a 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).

173. 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 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.

174. The nucleic acids that are delivered to cells typically contain expression 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 may contain upstream elements and response elements.

175. 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 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 (Fiers et al., *Nature*, 273: 113 (1978)). The immediate early promoter of the human cytomegalovirus is conveniently obtained as a *Hin*dIII E restriction fragment (Greenway, P.J. et al., *Gene* 18: 355-360 (1982)). Of course, promoters from the host cell or related species also are useful herein.

176. Enhancer generally refers to a sequence of DNA that functions at no fixed distance from the transcription start site and can be either 5' (Laimins, L. et al., *Proc. Natl. Acad. Sci.* 78: 993 (1981)) or 3' (Lusky, M.L., et al., *Mol. Cell Bio.* 3: 1108 (1983)) 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. Enhancers f unction 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 side of the replication origin, and adenovirus enhancers.

177. 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 chemotherapy drugs.

178. 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 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 LTF.

179. 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 melanoma cells. The glial fibrillary acetic protein (GFAP) promoter has been used to selectively express genes in cells of glial origin.

180. 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 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 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.

181. 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 ß-galactosidase, and green fluorescent protein.

182. 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, hydromycin, 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.

183. 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, (Southern P. and Berg, P., *J. Molec. Appl. Genet.* 1: 327 (1982)), mycophenolic acid, (Mulligan, R.C. and Berg, P. *Science* 209: 1422 (1980)) or hygromycin, (Sugden, B. et al., *Mol. Cell. Biol.* 5: 410-413 (1985)). 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.

184. 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.

185. 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.

186. Disclosed are methods of making a substance capable of modulating Stau1-mediated mRNA decay (SMD) activity, including but not limited to, modulation during the pioneer round, comprising admixing a substance identified by the methods of the invention with a pharmaceutically acceptable carrier. The invention also provides substances made by methods of the invention.

187. Disclosed are methods of making a modulator of Staul-mediated mRNA decay (SMD) comprising a) administering a substance to a system, wherein the system comprises SMD activity, b) assaying the effect of the substance on the amount of SMD activity in the system, c) selecting a substance which causes 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 substance, and d) synthesizing the substance.

188. 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: Human Upf1 Interacts With Human Staufen1

189. Using yeast two-hybrid analysis to screen a HeLa-cell cDNA library for encoded proteins that interact with full-length human Upf1, human Stau1 was identified in four out of one million transformants on the basis of growth at 37°C in galactose-containing medium but not glucose-containing medium (Figure 1A, upper). Galactose promoted transcription of cDNAs in the library, and the interaction of cDNA-encoded protein with Upf1 allowed for growth at 37°C. Sequence analysis of partial cDNAs that were obtained in the screen indicated that Upf1 interacts with Stau1 within the fourth double-stranded RNA binding domain, the tubulin binding domain, or both (Figure 1A, lower).

190. The ability of Upf1 to interact directly with Stau1 was confirmed using co-purification assays. First, GST-Upf1 that had been produced in E. coli interacted with 6xHis-Stau1 that had also been produced in E. coli. This was evident using GST pull-down to isolate GST-Upf1 (Figure 1B, upper) followed by either Western blotting using anti-His antibody (Figure 1B, middle) or Coomassie Blue staining (Figure 1B, lower) to confirm the presence of 6xHis-Stau1. Notably, 6xHis-Stau1 was not detected in the GST pull-down in the absence of GST-Upf1.

191. Second, FLAG-Upf1 that had been purified from HeLa cells as well as GST-Upf1 that had been purified from E. coli were shown by Far-Western analysis to interact with 6xHis-Stau1 or GST-Stau1 that had been produced in E. coli. To this end, each tagged Stau1

protein was electrophoresed in SDS-polyacrylamide and probed with either FLAG-Upf1 followed by Western blotting using anti-FLAG antibody (Figure 1C, middle) or GST-Upf1 followed by Western blotting using anti-GST antibody (Figure 1C, right). Coomassie Blue staining confirmed the presence of 6xHis-Stau1 and GST-Stau1 (Figure 1C, left).

192. Third, Stau1-HA_3 that had been immunopurified from Cos cells using anti-HA antibody co-purified with cellular Upf1 in a manner that was resistant to the addition of RNase A prior to immunopurification (IP) (Figure 1D, upper). This indicates that the interaction of Stau1 and Upf1 is stable in the absence of RNA. RNase A treatment was effective as demonstrated by the disappearance of cellular SMG7 mRNA in samples that were analyzed before IP (Figure 1D, lower). Stau1-HA_3 also co-immunopurified with Barentsz and CBP80, although in an RNase A-sensitive manner, but did not co-immunopurify with eIF4E or a non-specific control, Vimentin, which is a component of intermediate filaments (Figure 1D, upper). The co-IP of Stau1 with Barentsz had previously been shown to be RNase A-sensitive (Macchi et al., 2003). Since Barentsz is essential for EJC-dependent NMD (Palacios et al., 2004), and since CBP80 and the EJC are components of the pioneer translation initiation complex (Chiu et al., 2004; Ishigaki et al., 2001; Lejeune et al., 2002), Stau1 and Barentsz are also likely to be components of this complex. While antibodies to EJC components Upf3 and Upf3X immunopurified the EJC component Upf2, they failed to immunopurify either Stau1 or Barentsz (Figure 1E). These results indicate that: (i) the interaction of Stau1 and Barentsz with the EJC was destabilized by the experimental conditions, (ii) Stau1 and Barentsz only transiently interact with the EJC, as is the case for Upf1 (Ishigaki et al., 2001), or (iii) neither Stau1 nor Barentsz are components of the EJC.

193. Fourth, FLAG-Upf1 that had been isolated from HeLa cells using anti-FLAG antibody that was covalently conjugated to an agarose affinity gel (Pal et al., 2001) co-purified with both the 55-kDa and 63-kDa isoforms of Stau1 as well as Upf2 and Upf3X but not Vimentin (Figure 1F). These data also indicate that Stau1 and Upf1 are components of the same complex.

Example 2: Down-Regulating Cellular Stau1 Has No Detectable Consequence to the EJC-Dependent NMD of Gl or GPx1 mRNA

194. To gain insight into the possibility that Stau1 functions in EJC-dependent NMD, the effect of down-regulating the level of cellular Stau1 on the NMD of mRNAs for β-globin (Gl) and glutathione peroxidase (GPx)1 was examined using small interfering (si)RNAs. As a control, Upf3X was down-regulated in parallel. HeLa cells were transfected with Stau1

siRNA, Upf3X siRNA or a non-specific "Control" siRNA and, two days later, with three plasmids: (i) the pmCMV-Gl test plasmid that was either nonsense-free (Norm) or nonsense-containing (39Ter), (ii) the pmCMV-GPx1 test plasmid, either Norm or 46Ter, and (iii) the phCMV-MUP reference plasmid.

195. The level of each Stau1 isoform was down-regulated to 28% of normal, where normal is defined as the level in the presence of the non-specific Control siRNA, and the level of Upf3X was down-regulated to 24% of normal (Figure 2A). Using the level of Gl 39Ter or GPx1 46Ter mRNA as a measure of NMD, down-regulating Stau1 was of no detectable consequence to the NMD of either mRNA, whereas down-regulating Upf3X abrogated NMD 5-fold or 3-fold, respectively (Figure 2B). Therefore, Stau1 does not detectably function in the EJC-dependent NMD of nonsense-containing Gl and GPx1, which is consistent with the inability to detect Stau1 as a stable component of the EJC (Figure 1).

Example 3: Tethered Stau1 Reduces mRNA Abundance in a Mechanism that Involves Upf1 and an Upstream Nonsense Codon but Not Upf2 or Upf3X

196. In theory, Stau1 could elicit a type of EJC-independent mRNA decay based on the finding that it binds to Upf1. Previously, fusions of each Upf protein and the bacteriophage MS2 coat protein were shown to reduce mRNA abundance when tethered to a series of MS2 coat protein binding sites that were located more than 50 nts downstream of the normal termination codon (Lykke-Andersen et al., 2000). Since the reduced mRNA abundance was dependent on the normal termination codon, it was attributed to NMD. This or similar tethering methods were subsequently used to demonstrate that Y14, RNPS1, PYM and Barentsz also function in NMD (Bono et al., 2004; Gehring et al., 2003; Lykke-Andersen et al., 2001; Palacios et al., 2004).

197. Tethered Stau1 was similarly tested for the ability to reduce mRNA abundance depending on an upstream termination codon. HeLa cells were transiently transfected with three plasmids: (i) a pcFLuc test plasmid that produces Firefly (F) luciferase (Luc) from intronless FLuc cDNA that either did or did not harbor eight MS2 coat protein binding sites (MS2bs) within the 3' UTR (Figure 3A), (ii) the pRLuc reference plasmid that produces Renilla (R)Luc (Figure 3A), and (iii) a pMS2-HA or pMS2-HA-Stau1 effector plasmid that, respectively, harbored no insert so as to encode only MS2 coat protein (MS2)-HA or HA-Stau1 cDNA so as to encode a MS2-HA-Stau1 fusion protein.

198. Cells that had been transfected with pMS2-HA or pMS2-HA-Stau1 produced the expected fusion protein as evidenced by Western blotting using anti-HA antibody (Figure 3B,

left). As demonstrated using RT-PCR, while neither MS2-HA nor MS2-HA-Stau1 affected the level of FLuc mRNA that lacked the MS2bs, MS2-HA-Stau1 elicited a 3-to-4-fold reduction in the level of FLuc mRNA that harbored the MS2bs (Figure 3B, right). In control experiments that were performed in parallel, MS2-Upf1 and MS2-Upf2 (Lykke-Andersen et al., 2000) were also of no consequence to the level of FLuc mRNA that lacked the MS2bs but elicited a 2-to-5-fold reduction in the level of FLuc mRNA that harbored the MS2bs (Figure 3B, right). Furthermore, tethering an unrelated protein, HA-eIF4AIII, or expressing Stau-HA_3 or myc-Upf1 that could not be tethered failed to affect the abundance of FLuc-MS2bs mRNA (Figure 8).

199. These data are consistent with the possibility that tethering Stau1 downstream of a normal termination codon elicits mRNA decay. This possibility was examined in two ways. First, the effects of down-regulating the level of cellular Upf1, Upf2 or Upf3X on the Stau1-mediated reduction in FLuc-MS2bs mRNA abundance was examined. To this end, HeLa cells were transiently transfected with the appropriate siRNA and, two days later, with a combination of plasmids. One combination consisted of the pmCMV-Gl test plasmid, either Norm or 39Ter, and the phCMV-MUP reference plasmid. The other combination consisted of the pcFLuc-MS2bs test plasmid, the pRLuc reference plasmid, and an effector plasmid that produces MS2-HA, MS2-HA-Stau1, MS2, MS2-Upf1, MS2-Upf2 or MS2-Upf3.

200. Using Western blotting and the appropriate anti-Upf antibody, the level of Upf1 or Upf3X (Figure 5A, left) or Upf2 (Figure 5B, left) was shown to be down-regulated, respectively, to 4%, 28% or 13% of normal. In control experiments, down-regulating each protein abrogated the NMD of Gl 39Ter mRNA 3-to-5-fold (Figure 5A&B, middle), as expected. Down-regulating Upf1 but not Upf3X abrogated the decrease in the abundance of FLuc-MS2bs mRNA that was mediated by tethered Upf2 (Figure 5A, right). Furthermore, down-regulating Upf2 abrogated the decrease in the abundance of FLuc-MS2bs mRNA that was mediated by tethered Upf3 but had no effect on the decrease that was mediated by tethered Upf1 (Figure 5B, right). Therefore, tethering Upf2 obviates the involvement of cellular Upf3 or Upf3X but not Upf1 in EJC-dependent NMD, and tethering Upf1 obviates the involvement of the other Upf proteins in EJC-dependent NMD. It was conclude that Upf2 functions in NMD after Upf3 or Upf3X but before Upf1. Additionally, down-regulating Upf1 but not Upf3X or Upf2 abrogated the decrease in the abundance of FLuc-MS2bs mRNA that was mediated by tethered Stau1 (Figure 5A&B, right). It was concluded that Upf1 function obviates Stau1 function in the Stau1-mediated reduction in mRNA abundance, and that Stau1

reduces mRNA abundance in a mechanism that is not likely to involve Upf2, Upf3 or Upf3X. These results make sense given that Stau1 binds Upf1 (Figure 1), and that Upf1 is the last of the Upf proteins to function in EJC-dependent NMD (Lykke-Andersen et al., 2000).

201. In related experiments, the effect of down-regulating the level of cellular Stau1 or, as a control, cellular Upf1 on the NMD that was elicited by tethering Upf1, Upf2 or Upf3 to FLuc mRNA was determined. The level of each Stau1 isoform was down-regulated to 29% of normal, and the level of Upf1 was down-regulated to 7% of normal as evidenced using Western blotting (Figure 5C, left). According to expectations, down-regulating Upf1 abrogated the NMD that was elicited by tethering either Upf2 or Upf3 as evidenced using RT-PCR (Figure 5C, right). In contrast, however, down-regulating Stau1 had no effect on the NMD that was elicited by tethering Upf1, Upf2 or Upf3 (Figure 5C, right). These results are consistent with the finding that Stau1 does not detectably affect EJC-dependent NMD (Figure 2).

202. Moving the translation termination codon from a position that resides upstream of the MS2-HA-Stau1 tethering site to a position that resides downstream of the site was found to inhibit the Stau1-mediated reduction of both FLuc-MS2bs and Gl-MS2bs mRNA abundance (Figure 6). This result is consistent with the possibility that Stau1 reduces mRNA abundance by recruiting Upf1 to a position that resides downstream of a termination (i.e., nonsense) codon.

Example 4: Stau1 Binds the 3' UTR of Arf1 mRNA and Reduces the Abundance of mRNA Independently of an EJC

203. In search of substrates that could be natural targets of Stau1-mediated effects, ADP-ribosylation factor (Arf)1 mRNA, which encodes a Ras-related G protein that regulates membrane traffic and organelle structure (Donaldson and Jackson, 2000), was identified as a natural ligand of Stau1 using two methods. First, Stau1-containing RNPs were immunopurified from human 293 cells that transiently expressed either Stau1-HA_3 or, as a control for IP specificity, Stau1-6xHis using anti-HA antibody (Figure 7A). When biotin-labeled cRNAs were generated from the constituent RNAs and used to probe oligonucleotide arrays of 22,000 human genes, Arf1 mRNA was identified as one of at least 23 ligands of Stau1 (Figure 7B; Table 3). Second, using RT-PCR and a primer pair that specifically amplifies Arf1 mRNA, Arf1 mRNA was shown to be among the 293-cell transcripts that co-immunopurified with (i) anti-HA antibody in cells that expressed Stau1-HA_3 (Figure 7B) and (ii) anti-Stau1 antibody in untransfected cells (Figure 7C).

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 (*S. cerevisiae*)
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	*H. sapiens* transcribed sequences
LOC149603	AA085748	hypothetical protein LOC149603
TEGT	NM_003217	testis enhanced gene transcript (BAX inhibitor 1)
MCM4	AI859865	MCM4 minichromosome maintenance deficient 4 (*S. cerevisiae*)
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
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 immunopurified using anti-HA antibody and hybridized to Affymetrix U133A microarrays. Changes of at least 2.5-fold were scored as Stau1-interacting transcripts.

204. It was rationalized that if Stau1 binds to Arf1 mRNA at a position that reduces mRNA abundance, then down-regulating either Stau1 or Upf1 should up-regulate the level of Arf1 mRNA. Futhermore, down-regulating either Stau1 or Upf1 should fail to up-regulate the level of Arf1 mRNA that lacks the Stau1 binding site. To test these possibilities, HeLa cells were transiently transfected with Control siRNA or siRNA that down-regulates Stau1, Upf1, Upf2 or Upf3X. Two days later, cells were transiently transfected with two plasmids in order to measure effects on the abundance of Arf1 mRNA that was transiently produced from Arf1 cDNA. These plasmids consisted of: (i) a pSport-Arf1 or pSport-Arf1Δ(3'UTR) test plasmid, the latter of which lacks all nucleotides that reside downstream of the normal termination codon (Figure 8A), and (ii) the phCMV-MUP reference plasmid. Notably, the HeLa-cell Arf1

gene contains four introns (Lee et al., 1992; Figure 8A) so that the resulting newly synthesized mRNA would harbor four EJCs. In contrast, since Arf1 cDNA within either pSport plasmid lacks all introns, the resulting newly synthesized mRNA would lack EJCs.

205. The results of Western blotting demonstrated that the level of each Stau1 isoform was down-regulated to 26% of normal, and the level of Upf1, Upf2 or Upf3X was down-regulated to, respectively, 21%, 22% or 13% of normal (Figure 8B, upper). The results of RT-PCR using RNA from the same cells demonstrated that the abundance of endogenous HeLa-cell Arf1 mRNA relative to the abundance of SMG7 as well as the abundance of Arf1 mRNA that derived from Arf1 cDNA relative to MUP mRNA was increased 2-to-4-fold in the presence of Stau1 or Upf1 siRNA relative to Control siRNA (Figure 8B, second and third down; see also Figure 9 for comparable results using a different Stau1 or Upf1 siRNA). In contrast, and as expected from the Stau1 tethering results (Figures 3 and 5), the abundance of Arf1 mRNA from the gene or cDNA was unaffected by down-regulating Upf3X (Figure 8B, second and third down). For reasons that are not entirely clear, the abundance of Arf1 mRNA was decreased by down-regulating Upf2 (Figure 8B, second and third down). This decrease may simply reflect competition between Stau1 and Upf2 for binding to Upf1. Possibly, down-regulating Upf2 augments the interaction of Stau1 with Upf1, which could increase the efficiency of NMD. Consistent with this interpretation, Upf2 is not detected in association with Stau1, whereas both Upf2 and Stau1 are detected in association with Upf1 (Figure 1F). However, down-regulating Upf2 did not augment NMD when Stau1 was tethered (Figure 5B). These data indicate that Stau1 reduces the abundance of Arf1 mRNA in a mechanism that involves Upf1 but not Upf2 or Upf3X.

206. These findings also indicate that Stau1 binds Arf1 mRNA at a position that is located within the 3' UTR. Consistent with this, deletion of the 3' UTR increased the level of Arf1 mRNA 7-fold (Figure 8C). Moreover, in contrast to Arf1 mRNA, the abundance of Arf1Δ(3'UTR) mRNA was unaffected by Stau1 siRNA relative to Control siRNA (Figure 8C).

207. In order to test for Stau1 binding to the 3' UTR of Arf1 mRNA, Cos cells were transiently transfected with the Stau1-HA_3 expression vector, the pSport-Arf1 or pSport-Arf1Δ(3'UTR) test plasmid, and the phCMV-MUP reference plasmid. Notably, cells were transfected with only half as much pSport-Arf1Δ(3'UTR) as pSport-Arf1 in order to compensate for the difference in the level of product mRNA. Cell extract was prepared two days later, and a fraction was immunopurified using anti-HA antibody or, as a control for

nonspecific IP, rat (r) IgG. Western blotting of immunopurified protein demonstrated that the efficiency of IP was 11% (Figure 8D, upper). RT-PCR of immunopurified RNA demonstrated the presence of Arf1 mRNA, a low level of Arf1Δ(3'UTR) mRNA, and no detectable MUP mRNA (Figure 8D, lower). It was concluded that Stau1 binds to the 3' UTR of Arf1 mRNA, as was predicted from data demonstrating that Arf1 mRNA but not Arf1Δ(3'UTR) mRNA is naturally targeted for a Stau1-mediated reduction in abundance. Additionally, it was concluded that Stau1 also binds elsewhere within Arf1 mRNA, albeit to a lesser extent. Consistent with this conclusion, and in support of the idea that a termination codon must reside upstream of a Stau1 binding site in order for Stau1 to mediate a reduction in mRNA abundance (Figure 6), 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. It follows that Stau1 binds between codon 35 and the normal termination codon with less efficiency than it binds to the 3' UTR. Notably, the specificity of Stau1 binding to Arf1 mRNA was illustrated by the failure of Stau1 to bind to MUP mRNA.

208. Since we now know that Stau1 binding to mRNA can lead to mRNA decay, the transcripts that we reported in Table 3, can be weak targets for decay. Alternatively, the transcripts can be up-regulated by Stau1 binding. To determine what transcripts are up-regulated and which are down-regulated after down-regulating Stau1, microarray analysis was performed (Tables 4 and 5).

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 precusor [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

Table 5. Transcripts down-regulated after down-regulating Staul in two independently performed analyses

(Note : Redundancies reflect different wells in each microarray)

213017_at	abhydrolase domain containing 3

237159_x_at	adaptor-related protein complex 1 sigma 3 subunit
208030_s_at	adducin 1 (alpha) /// adducin 1 (alpha)
225711_at	ADP-ribosylation-like factor 6-interacting protein 6
1555854_at	aldo-keto reductase family 1 member C1 (dihydrodiol dehydrogenase 1; 20-alpha (3-alpha)-hydroxysteroid dehydrogenase)
225229_at	ALL1 fused gene from 5q31
204288_s_at	Arg/Abl-interacting protein ArgBP2
242037_at	aspartate beta-hydroxylase
203192_at	ATP-binding cassette sub-family B (MDR/TAP) member 6
200756_x_at	calumenin
210735_s_at	carbonic anhydrase XII
1569263_at	casein kinase 1 delta
221156_x_at	cell cycle progression 8 protein
222552_at	CGI-141 protein
230151_at	chromosome 13 open reading frame 1
208763_s_at	delta sleep inducing peptide immunoreactor
223772_s_at	DKFZP564G2022 protein
202972_s_at	family with sequence similarity 13 member A1
202916_s_at	family with sequence similarity 20 member B
223321_s_at	fibroblast growth factor receptor-like 1
229908_s_at	GlcNAc-phosphotransferase gamma-subunit
218839_at	hairy/enhancer-of-split related with YRPW motif 1
1568598_at	Homo sapiens cDNA clone IMAGE:6094109 partial cds
226822_at	Homo sapiens cDNA FLJ10532 fis clone NT2RP2001044.
232060_at	Homo sapiens cDNA FLJ20769 fis clone COL06674
243940_at	Homo sapiens hypothetical gene supported by NM_018692 (LOC374296) mRNA
228141_at	Homo sapiens mRNA; cDNA DKFZp686G03142 (from clone DKFZp686G03142)
244359_s_at	Homo sapiens transcribed sequences
228437_at	HSPC163 protein
225867_at	hypothetical protein BC013767
228574_at	hypothetical protein DKFZp762A217
219383_at	hypothetical protein FLJ14213
204508_s_at	hypothetical protein FLJ20151
226600_at	hypothetical protein FLJ90492
226604_at	hypothetical protein FLJ90492
232275_s_at	hypothetical protein LOC283476
205227_at	interleukin 1 receptor accessory protein
229139_at	junctophilin 1
205206_at	Kallmann syndrome 1 sequence
212792_at	KIAA0877 protein
212942_s_at	KIAA1199 protein
232155_at	KIAA1618 protein
1561180_at	low density lipoprotein receptor-related protein 11
212713_at	microfibrillar-associated protein 4
32625_at	natriuretic peptide receptor A/guanylate cyclase A (atrionatriuretic peptide receptor A)
200732_s_at	protein tyrosine phosphatase type IVA member 1

225207_at	pyruvate dehydrogenase kinase isoenzyme 4
205960_at	pyruvate dehydrogenase kinase isoenzyme 4
205087_at	RWD domain containing 3
223843_at	scavenger receptor class A member 3
225123_at	sestrin 3
235150_at	sestrin 3
242943_at	sialyltransferase 8D (alpha-2 8-polysialyltransferase)
217859_s_at	solute carrier family 39 (zinc transporter) member 9
227031_at	sorting nexin 13
202991_at	START domain containing 3
207320_x_at	staufen RNA binding protein (Drosophila)
211505_s_at	staufen RNA binding protein (Drosophila)
213037_x_at	staufen RNA binding protein (Drosophila)
208948_s_at	staufen RNA binding protein (Drosophila)
209131_s_at	synaptosomal-associated protein 23kDa
201009_s_at	thioredoxin interacting protein
201010_s_at	thioredoxin interacting protein
201008_s_at	thioredoxin interacting protein
209295_at	tumor necrosis factor receptor superfamily member 10b
227812_at	tumor necrosis factor receptor superfamily member 19
221291_at	UL16 binding protein 2
205139_s_at	uronyl-2-sulfotransferase
227278_at	Unknown transcript
1568513_x_at	Unknown transcript (don't know if same as above)

209. 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 Staufen1 or Upf1 but not Upf2 increased the abundance of each cellular mRNA. Notably, the abundance of many transcripts was down-regulated in the cells depleted of Stau1. These results indicate that Stau1 can mediate the an increase in the abundance of some transcripts and also a decrease in the abundance of other transcripts.

Example 5: A Minimized Stau1 Binding Site Resides ≥ 67 Nucleotides Downstream of the Normal Termination Codon of Arf1 mRNA and Mediates mRNA Decay

210. To further localize the Staul binding site within the Arfl 3' UTR, a series of deletions was generated from the distal-most nucleotide of the Arfl 3' UTR within pSport-Arfl (Figure 10A). Each of the resulting test plasmids was transiently introduced into 293 cells together with the Stau-HA_3 expression vector. Cell extract was prepared two days later, and a fraction was immunopurified using anti-HA antibody. Western blotting of immunopurified protein revealed relative IP efficiencies (Figure 10B, upper). RT-PCR of immunopurified RNA demonstrated that Staul binds between nucleotides 689 and 919 (Figure 10B, lower, where the first transcribed nucleotide of endogenous Arfl mRNA is defined as 1). Notably, the small amount of Staul binding that is detected using mRNAs that terminate at nucleotide 621 or 688 reflects the finding that Staul binds less efficiently to the coding region than to the 3' UTR (Figure 10D). While these data do not rule out the possibility that Staul also binds within the 3' UTR downstream of nucleotide 919 or upstream of nucleotide 688, the finding that inserting the Staul binding site (SBS, nucleotides 622-924) within the 3' UTR of the heterologous FLuc mRNA reduced FLuc mRNA abundance (Figure 11) corroborates that Staul indeed binds between nucleotides 689 and 919. It was concluded that Staul binds to the 3' UTR of Arfl mRNA at a position that is predicted to reduce mRNA abundance.

211. To determine if Staul mediates a reduction in Arfl mRNA half-life, the expression of Arfl cDNA harboring the SBS in place of the full-length 3' UTR was driven by the fos promoter (Figure 10C, upper). This promoter is transiently inducible upon the addition of serum to serum-deprived cells and, thus, provides a way to analyze mRNA half-life (Lejeune et al., 2003). L cells were transfected with mouse (m)Staul or Control siRNA and, two days later, with the pfos-Arfl-SBS and the phCMV-MUP reference plasmid in the absence of serum. One day later, serum was added, and protein and RNA were purified from, respectively, cytoplasmic and nuclear fractions at 0, 30, 60, 90 and 120 min. Western blotting demonstrated that the level of mStaul was down-regulated to 21%, 18%, and 26% of normal (Figure 10C, second-from-top, left and right). RT-PCR demonstrated that down-regulating mStaul increased the half-life of nucleus-associated fos-Arfl-SBS mRNA (Figure 10C, second-from-bottom, left and right). These data demonstrate that Staul mediates the nucleus-associated decay of Arfl mRNA, which is called Staul-mediated mRNA decay (SMD). The specificity of the SBS in mediating SMD was evident with the finding that down-regulating Staul to 29% increased the level of fos-FLuc-SBS (Figure 11B), but down-regulating Stual to 212% had no effect on fos-Arfl (Δ3'UTR) mRNA

(Figure 13), which is consistent with the increase evident after the addition of serum to serum-deprived cells, but did not increase the level of fos-Arf1(Δ3' UTR). This shows that Stau1 and Upf1 mediate the instability of fos-Arf1 mRNA in a way that depends on the Arf1 3' UTR. Down-regulating mUpf1 relative to normal also increased the half-life of nucleus-associated fos-Arf1-SBS mRNA (ie., abrogated the nucleus associated NMD), whereas down-regulating mUpf2 or mUpf3X relative to normal did not. Furthermore, down-regulating mStau1 and mUpf1 also increased the half-life of a heterologous mRNA (fos-FLuc-Arf1) harboring the SBS, but had no effect on fos-FLuc-Arf1 without the SBS. These data, like steady-state data (Figures 5, 8, 9, 11B) indicate that SMD involves Upf1 but not Upf2 or Upf3X.

212. In this disclosure, a novel role for Stau1 and new type of mRNA decay that does not involves Upf1 but not an EJC was described. It was demonstrated that mammalian Stau1 directly binds the NMD factor Upf1 (Figure 1). It was also demonstrated that tethering Stau1 downstream of a termination codon reduces mRNA abundance (Figure 3) in a mechanism that appears to involve the termination codon (Figure 6) and Upf1 but not Upf2 or Upf3X (Figure 5). This mechanism is shown to be physiologically relevant with the finding that Stau1 binds to an ~230-nt region of the 3' UTR of Arf1 mRNA so as to recruit Upf1 independently of other Upf proteins and, as a consequence, reduce Arf1 mRNA half-life (Figures 7, 8, 9, 10, 11).

213. It was found that Stau1 plays no detectable role in the EJC-dependent NMD of Gl and GPx1 mRNAs (Figure 2). This is despite the ability of Stau1 to interact with Barentsz (Macchi et al., 2003; Figure 1), which interacts directly with the EJC component eIF4AIII (Chan et al., 2004; Palacios et al., 2004). However, neither Stau1 nor Barentsz is detected as a stable component of the EJC (Figure 1).

214. 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. In fact, Stau1 binds the 3' UTR of PAICS mRNA (and also elsewhere within the mRNA), and down-regulating Stau1 increases PAICS mRNA abundance ~2-fold (Figure 12). Notably, natural substrates for SMD could, in theory, arise by

alternative splicing, which has been proposed to result the generation of a premature termination codon one-third of the time (Hillman, RT et al., 2004). Furthermore, those Stau1-binding mRNAs that are also products of pre-mRNA splicing will additionally be subject to EJC-dependent NMD if they terminate translation a sufficient distance upstream of an EJC.

215. As a rule, a termination codon that resides more than ~50-55 nt upstream of an exon-exon junction elicits NMD (Nagy and Maquat, 1998). Considering that an EJC resides ~20-25 nts upstream of an exon-exon junction (Le Hir et al., 2000a), it follows that a termination codon that resides more than ~25 nt upstream of a Stau1 binding site should elicit SMD. These two scenarios are exemplified for Arf1 mRNA, which harbors four exon-exon junctions and binds Stau1 ≥ 67 nt downstream of the normal termination codon (Figure 14). In theory, it may be possible for a termination codon to be situated so that it elicits both EJC-independent and EJC-dependent NMD. In fact, the data indicate that this possibility exists for Arf1 mRNA, which is normally a product of pre-mRNA splicing and binds Stau1 within its coding region as evidenced by the ability of 35Ter within Arf1 cDNA to elicit SMD.

216. To date, there are a number of RNP proteins that function in two distinct RNA metabolic processes, one of which involves mRNA localization coupled to translational control. As one example, Hrb27C inhibits the removal by nuclear splicing of intron 3 from P-element encoded transposase pre-mRNA in Drosophila (Hammond et al., 1997; Siebel et al., 1994) and also binds directly to Drosophila gurken mRNA so as to regulate gurken mRNA cytoplasmic localization and translation (Goodrich et al., 2004). As other examples, Barentsz and Y14, the latter of which is a component of the EJC (Kim et al., 2001; Lau et al., 2003; Palacios et al., 2004), are essential for EJC-dependent NMD in mammalian cells (Ferraiuolo et al., 2004; Gehring et al., 2003; Palacios et al., 2004; Shibuya et al., 2004) and also function in localizing Drosophila oskar mRNA to the posterior pole of the oocyte (Hachet and Ephrussi, 2001; Hachet and Ephrussi, 2004; Mohr et al., 2001; van Eeden et al., 2001). Similarly, it was shown here that mammalian Stau1, which is a component of RNPs that are transported and localized within dendrites of mature hippocampal neurons (Kiebler et al., 1999; Kohrmann et al., 1999), is involved in a new type of EJC-independent NMD.

217. In mammals, Stau1 is ubiquitously expressed, but its level of expression varies among tissues (Wickham et al., 1999; Marion et al., 1999; Monshausen et al., 2001). For example, it is highly expressed in brain, heart, liver, testis, pancreas and placenta, whereas it is generally expressed to lesser but varying degrees in other tissues. Additionally, differential splicing generates several isoforms that are not uniformly expressed among

tissues. Stau1 also contains several putative phosphorylation sites that could regulate function, and Stau1 has been shown to interact with protein phosphate 1 (Monshausen et al., 2002). Since Upf1 phosphorylation influences Upf1 function in NMD (Pal et al., 2001; Ohnishi et al., 2003, it is possible that Upf1 phosphorylation also regulates its function or the function of Stau1 in SMD. Therefore, Stau1 functions can be modulated in different tissues according to the level of expression and the nature of each expressed isoform and, possibly, the degree of Upf1 or Stau1 phosphorylation.

218. Stau1 is linked to mRNA transport in neurons (Kohrmann et al., 1999) and to HIV-1 replication (Mouland et al., 2000). Its association with polysomes (Marion et al., 1999; Luo et al., 2002) suggests a role in the translational regulation Stau1-bound mRNAs. Furthermore, biochemical and proteomic analyses demonstrate that Stau1 is a component of large RNA granules (Krishevsky and Kosik, 2001; Kanai et al., 2004; Mallardo et al., 2003; Villace et al., 2004, Brendel et al., 2004; Ohashi et al., 2002) that contain ribosomes, mRNA and several other proteins. It is shown herein that several Stau1-bound mRNAs encode key regulatory enzymes that control cell metabolism, proteins involved in organelle trafficking, cell division or the cell cycle, or both. Arf1 is involved in protein trafficking, and it may modulate vesicle budding and uncoating within the Golgi apparatus. PAICS controls steps 6 and 7 of the purine nucleotide biosynthetic pathway. Interestingly, the level of PAICS mRNA varies with the cell cycle in synchronized rat 3Y1 fibroblasts (Iwahana et al., 1995). This variation can be controlled by SMD. GNE is a regulatory enzyme that regulates the first step in the biosynthesis of N-acetylneuraminic acid (NeuAc), which is a precursor to sialic acids (Hinderlich et al., 1997). Modification of cell surface molecules with sialic acid is essential for many biological processes, such as cell adhesion and signal transduction. Modulation of sialylation is involved in the tumorigenicity and metastatic behavior of malignant cells. While the activity of GNE and its gene are regulated, the data indicates that the level of GNE mRNA can also be regulated by SMD. From these and other examples, it is recognized that modulation of Stau1, Upf1 or both through modulation in the level of isoform expression, post-translational modification, or both can control the level of SMD in response to signal transduction or other regulatory pathways.

219. In summary, the findings expand the role of Stau1 in post-transcriptional gene control. Significant issues that have been brought to light by these studies include if the Stau1-mediated recruitment of Upf1 to mRNAs impacts mammalian development, neuronal

transmission, or cellular growth control. It will also be important to determine if Staufen and Upf1 interact in Drosophila so as to elicit mRNA decay.

Example 6: Plasmid Constructions

220. To construct the pSos-Upf1 yeast two-hybrid bait plasmid, the HindIII/Klenow-filled NotI fragment that contains human UPF1 cDNA from pCI-neo-FLAG-UPF1 (Sun et al., 1998) was inserted into the NotI/Klenow-filled SalI fragment from pSos (Stratagene).

221. For the bacterial production of human Upf1 that harbors an N-terminal GST tag, pGEX-UPF1 was constructed by ligating the NotI/Klenow-filled EcoRI fragment from pCMV-Myc-UPF1 that contains UPF1 cDNA to the NotI/Klenow-filled BamHI fragment from pGEX-6p-1+NdeI (a gift from J. Wedekind).

222. For the bacterial production of human Staufen1 (Stau1) that harbors an N-terminal GST tag, pGEX-Stau1 was constructed by ligating the BamHI/NotI fragment from pGEX-6p-1+NdeI to a PCR-amplified fragment that had been digested with BglII and NotI. The PCR fragment was amplified using the human Stau1 cDNA expression vector phStau1^{55}-HA_3 (Luo et al., 2002) and two primers: 5'-GAAGATCTAGAATGAAACTTGGAAAAAAACCAATGTATAAG-3' (SEQ ID NO. 5) (sense) and 5'-ATAAGAATGCGGCCGCTCAGCACCTCCCACACACAGACATTGGTCC-3' (SEQ ID NO. 6) (antisense), where underlined nucleotides specify the BglII or NotI site, respectively.

223. For the bacterial production of human Stau1 that harbors an N-terminal 6xHis tag, pRSET B-Stau1 was constructed by ligating the Klenow-filled NdeI/NotI fragment from pGEX-Stau1 to the Klenow-filled BglII fragment from pRSET B (Invitrogen).

224. To construct pMS2-HA-Stau1 that encodes N-terminal oligomerization-defective MS2 coat protein followed successively by an HA tag and full-length human Stau1, pCI-neo (Promega) that had been digested with NheI and NotI was ligated to two fragments: a PCR-amplified fragment that contains the MS2 coat protein-encoding sequence that had been digested with NheI and BamHI, and a PCR-amplified fragment that contains human Stau1 cDNA that had been digested with BglII and NotI. The MS2 coat protein-encoding fragment was amplified using pET-MS2 (Coller et al., 1998) and two primers: 5'-CGCTACTAGCTAGCCGCCATGGCTTCTAACTTTACTCAGTTCGTTC-3' (SEQ ID NO. 7) (sense) and 5'-ATAAGAATGCGGCCGCCTAGGATCCAGCGTAGTCTGGAACGTCGTATGGGTAGA TGCCGGAGTTTGCTGCGATTG-3' (SEQ ID NO. 8) (antisense), where underlined and

bold nucleotides specify the NheI or NotI site and HA tag sequence, respectively. The Stau1 cDNA-containing sequence was amplified using phStau1[55]-HA_3 and two primers: 5'-GAAGATCTAGAATGAAACTTGGAAAAAAACCAATGTATAAG-3' (SEQ ID NO. 5) (sense) and 5'-ATAAGAATGCGGCCGCTCAGCACCTCCCACACACAGACATTGGTCC-3' (SEQ ID NO. 6) (antisense), where underlined nucleotides specify the BglII or NotI site, respectively.

225. To construct pMS2-HA that harbors an N-terminal MS2 coat protein followed by an HA tag, pCI-neo that had been digested with Nhe1 and Not1 was ligated to the same PCR-amplified fragment that contains the MS2 coat protein-encoding sequence and was similarly digested with Nhe1 and Not1 (see above).

226. pcFLuc-MS2bs, which contains eight tandem repeats of the MS2 coat protein-binding sites within the 3'UTR of firefly luciferase (FLuc) cDNA, was generated from pcβ-8bs (Lykke-Andersen et al., 2000) by replacing β-globin cDNA with FLuc cDNA. pcFLuc, which lacks the MS2 coat protein-binding sites, was generated from pcFLuc-8bs by cleaving with PspOMI and NotI followed by self-ligation after generating blunt ends using Klenow (New England Biolabs).

227. pRLuc, which encodes renilla luciferase (RLuc), was generated from p2luc (Grentzmann et al., 1998) by precisely deleting FLuc cDNA.

228. Full-length Arf1 cDNA was obtained by RT-PCR (RNA PCR Core Kit, Applied Biosystems) using total HEK293-cell RNA and two primers: 5'-AGGTCTAGATCGGAGCAGCAGCCTCTGAGGTGT-3' (SEQ ID NO. 9) (sense) and 5'-GGCTCGAGCTAATAGCTATAATTACAGTGCTTGTTTGTCGAAATG-3' (SEQ ID NO. 10) (antisense), where underlined nucleotides specify the XbaI and XhoI site, respectively. The PCR product was digested with XbaI and XhoI and ligated to pSport ß-gal (Invitrogen) that had been digested with XbaI and XhoI. The resulting plasmid, pSport ß-gal-Arf1, was digested with NotI, purified, and self-ligated to generate pSport-Arf1.

229. To construct pSport-Arf1Δ(3'UTR), pSport-Arf1 was digested with KpnI and XhoI. The resulting vector-containing fragment was ligated to a PCR-amplified fragment that contains the Arf1 open translational reading frame and had been digested with KpnI and XhoI. PCR was carried out using pSport-Arf1 and two primers: 5'-GCTATTTAGGTGACACTATAGAAGGTAC-3' (SEQ ID NO. 11) (sense) and 5'-CCGCTCGAGTTCACTTCTGGTTCCGGAGCTGATTG-3' (SEQ ID NO. 12) (antisense), where underlined nucleotides specify the HindIII site.

230. Deletions within the 3' UTR of Arf1 cDNA were generated using pSport-Arf1, 5'-CATTTCGACAAACAAGCACTGTAATTATAGCTATTAG-3' (SEQ ID NO. 13) (sense) and one of the following antisense primers: 5'-CCAAGGACAAGCGAGTTGCG-3'(Δ1229-1794) (SEQ ID NO. 14), 5'-CGACTGGCATCCAGGCCGTAAC-3'(Δ1123-1794) (SEQ ID NO. 15), 5'-GTGCCCATGGGCCTACATCC-3'(Δ 920-1794) (SEQ ID NO. 16), 5'-ACGAGCCGCACGTTTGCCA-3'(Δ689-1794) (SEQ ID NO. 17), 5'-GTTCACTTCTGGTTCCGGAGCTG-3'(Δ622-1794) (SEQ ID NO. 18). PCR amplifications were carried out using Pfu Ultra (Stratagene). PCR products were incubated with DpnI to digest the methylated template DNA, phosphorylated at the 5' ends using T4 polynucleotide kinase (Fermentas), and circularized by ligation.

231. To construct pfos-Arf1-SBS, pfos-Gl (Lejeune et al., 2004) was digested with NcoI and EcoRI and ligated to a PCR-amplified fragment that contains Arf1-SBS cDNA. PCR was carried out using pSport-Arf1 and two primers : 5'-ACAACCATGGGGAACATCTTCGCCAACCTCTTC-3' (sense) (SEQ ID NO. 19) and 5'-CCGGAATTCTGGGCCTACATCCCCTCTCAGCACTGAAC-3' (SEQ ID NO. 20) (antisense), where underlined nucleotides specify the NcoI or EcoRI site.

232. To construct pMS2-HA-eIF4AIII, which encodes N-terminal oligomerization-defective MS2 coat protein followed successively by an HA tag and full-length human eIF4AIII cDNA, pCI-neo that had been digested with NheI and NotI was ligated to two fragments: the NheI/BamHI fragment from pMS2-HA-Stau1 that contains the MS2 coat protein-encoding sequence, and a PCR-amplified fragment that contains human eIF4AIII cDNA and had been digested with BamHI and NotI. eIF4AIII was amplified using pcDNA3-HA-eIF4AIII (Chiu et al., 2004) and two primers: 5'-CGCGGATCCATGGCGACCACGGCCACGATGGCGACC-3' (SEQ ID NO. 21) (sense) and 5'-ATAAGAATGCGGCCGCTCAGATAAGATCAGCAACGTTCATCGG-3' (SEQ ID NO. 22) (antisense), where underlined nucleotides specify the BamHI or NotI site, respectively.

233. To construct pcFLuc(UAA→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 (Kim et al., 2003) and two primers: 5'-TTGACCGCTTGAAGTCTTTAATTAAATAC-3' (SEQ ID NO. 23) (sense) and 5'-

CGAAGCGGCCGCAATTACATTTTGCAATTTGGACTTTCCGCCCTTCTTGGC-3' (SEQ ID NO. 24) (antisense). Underlined nucleotides specify a NotI site.

234. To construct pcFLuc-Arf1 SBS or pcFLuc-Arf1 No SBS, pcFLuc-8bs were digested with XbaI and ligated to a XbaI-digested PCR-amplified fragment that had been generated using pSport-Arf1 and two primers: 5'-GCTCTAGAGTGACCGAATTCGTGAACGCGACCCCCCTCCCTCTCACTC-3' (SEQ ID NO. 25) (sense) and 5'-GCTCTAGAGGGCCCAGGTGCCCATGGGCCTACATCCCC-3' (SEQ ID NO. 26) (antisense) for pcFLuc-Arf1 SBS, or 5'-GCTCTAGAGTGACCGAATTCGTGAGAGGGGATGTAGGCCCATGGGCAC-3' (SEQ ID NO. 27) (sense) and 5'-GCTCTAGAGGGCCCGAGGGGAACAGCTGGGCTGGCGACTGG-3' (SEQ ID NO. 28) (antisense) for pcFLuc-Arf1 No SBS. Underlined nucleotides specify XbaI sites.

235. To construct pfos-Arf1-SBS or pfos-Arf1-No SBS, pfos-Gl (Lejeune et al., 2004) was digested with NcoI and EcoRI and ligated to a PCR-amplified fragment that contains Arf1-SBS cDNA or Arf1-No SBS cDNA. PCR was carried out using pSport-Arf1 and two primers : 5'-ACAACCATGGGGAACATCTTCGCCAACCTCTTC-3' (sense) (SEQ ID NO: 57) and 5'-CCGGAATTCTGGGCCTACATCCCCTCTCAGCACTGAAC-3' (antisense) (SEQ ID NO: 58) for Arf1-SBS cDNA or 5'-CCGGAATTCTCACTTCTGGTTCCGGAGCTGATTGGAC-3' (SEQ ID NO: 59) (antisense) for Arf1-No SBS cDNA, where underlined nucleotides specify the NcoI or EcoRI site.

236. pSport-PAICS was purchased from ATCC (catalog # MGC-5024, NCBI Accession # BC010273). pSport-PAICS Δ(3'UTR) was generated using pSport-PAICS and two primers: 5'-CCAAGCTTACGCGTACCCAGCTTTC-3' (SEQ ID NO. 29) (sense) and 5'- CCCCTAAAAAATTCAATGGCATTCTTTC-3' (SEQ ID NO. 30) (antisense). PCR amplification was carried out using Pfu Ultra (Stratagene). The PCR product was incubated with DpnI to digest the methylated template DNA, phosphorylated at the 5' ends using T4 polynucleotide kinase (Fermentas), and circularized by ligation.

237. pCDNA3-RSV-CK2A2 was constructed by inserting a BamHI-XhoI fragment from pOTB7-CK2A2 (ATCC catalog # MGC-10397, NCBI Accession # BC008812) into pCDNA3-RSV that had been digested using BamHI and XhoI.

Example 7: Yeast Two-Hybrid Analysis

238. Proteins that interact with human Upf1 were identified using the CytoTrap Two-Hybrid System (Stratagene). The yeast strain cdc25H was transformed with pSos-Upf1 and the pMyr library of HeLa-cell cDNAs (Stratagene). Transformants were processed following manufacturer instructions.

Example 8: siRNA-Mediated Down-Regulation of Human Upf1, Upf2, Upf3X or Stau1

239. HeLa cells (2 x 10^6) were grown in DMEM medium (Gibco-BRL) containing 10% fetal bovine serum (Gibco-BRL) in 60-mm dishes and transiently transfected with 100 nM of in vitro-synthesized small interfering (si)RNA (Xeragon or Dharmacon) using Oligofectamine (Invitrogen). Upf1, Upf2, Upf3X or Stau1 were down-regulated using, respectively, 5'-r(GAUGCAGUUCCGCUCCAUU)d(TT)-3' (SEQ ID NO. 31), 5'-r(GGCUUUUGUCCCAGCCAUC)d(TT)-3' (SEQ ID NO. 32), 5'-r(GGAGAAGCGAGUAACCCUG)d(TT)-3' (SEQ ID NO. 33), or 5'-r(CCUAUAACUACAACAUGAG)d(TT)-3' (SEQ ID NO. 34).

240. In experiments that involved siRNA and tethering (see below), cells were re-transfected 48 h later with the specified reporter, effector, and reference plasmids. Cells were harvested two days later. To test for the abrogation of Gl, GPx1 or Arf1 NMD, cells were re-transfected 48 h after siRNA introduction with (i) 0.05 μg of a pmCMV-Gl test plasmid, either nonsense-free (Norm) or nonsense-containing (39Ter) (Zhang et al., 1998), (ii) or 0.05 μg of a pmCMV-GPx1 test plasmid, either Norm or 46Ter (Moriarty et al., 1998), (iii) 0.05 μg of a pSport-Arf1 or pSport-Arf1Δ(3'UTR) test plasmid, and (v) 0.1 μg of the phCMV-MUP reference plasmid (Belgrader and Maquat, 1994) using Lipofectamine Plus Reagent (Invitrogen).

241. Stau1(A) siRNA consisted of 5'-r(GUUUGAGAUUGCACUUAAA)d(TT)-3' (SEQ ID NO. 35), and Upf1(A) siRNA consisted of 5'-r(AACGUUUGCCGUGGAUGAG)d(TT)-3' (SEQ ID NO. 36).

242. Alternatively, in microarray experiments that involved the specific down regulation of Stau1, HeLa cells (5 x 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. 34). 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.

Example 9: Tethering Experiments

243. HeLa cells (2 x 10^6) were transiently transfected using Lipofectamine Plus (Invitrogen) with 0.3 μg of the reporter plasmid pcFLuc or pcFLuc-MS2bs, 0.02 μg of the reference plasmid pRLuc, and 5 μg 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 10: fos Promoter Induction Experiments

244. Ltk$^-$ cells (4 x 10^6) were transiently transfected with 200 nM of Control siRNA or 100 nM each of two different mouse Stau1 siRNAs [5'-r(CAACUGUACUACCUUUCCA)d(TT)-3' (SEQ ID NO. 37) or 5' r(AACGGUAACUGCCAUGAUA)d(TT)-3' (SEQ ID NO. 38)]. Alternatively, Upf1, Upf2 or Upf3X siRNA was used [5'-r(UCAAGGUUCCUGAUAAUUA)dTT-3' (SEQ ID NO: 60), 5'-r(GAAACUUCUUGAUGAACAA)dTT-3' (SEQ ID NO: 61), or 5'-r(AGAGGCACUUCGAGAUAAA)dTT-3'(SEQ ID NO: 62), respectively]. Two days later, cells were retransfected with 0.3 μg of the pfos-Arf1-SBS test plasmid, and the phCMV-MUP reference plasmid as described above. The fos promoter was transiently induced, and cells were fractionated as described (Lejeune et al., 2003). However, serum was eliminated from the second transfection and added back one day later.

Example 11: Immunopurifications

245. Cos-7 or HeLa cells were cultured as described above but in 150-mm dishes. Transfections and immunopurifications (IPs) were performed as described (Chiu et al., 2004; Ishigaki et al., 2001; Lejeune et al., 2002).

Example 12: Western Blotting

246. Protein was electrophoresed in SDS-polyacrylamide, transferred to Hybond ECL nitrocellulose (Amersham), and probed with antibodies that recognize FLAG (Sigma), GST (Qiagen), HA (Roche), Upf1 (Lykke-Andersen et al., 2000), Upf2 (Serin et al., 2001), Upf3/3X (Serin et al., 2001), Barentsz (Macchi et al., 2003), CBP80 (Izaurralde et al., 1994), eIF4E (Santa Cruz), Vimentin (Santa Cruz), human Stau1 (Wickham et al., 1999), mouse Stau1 (Marion et al., 1999), PABP1 (Gorlach et al., 1994), PABP2 (Krause et al., 1994), or

eIF3b (a gift from N. Sonenberg) as described (Chiu et al., 2004; Lejeune et al., 2002; Lejeune et al., 2003).

Example 13: RT-PCR

247. The levels of specific RNAs were quantitated using RT-PCR as described (Sun et al., 1998). FLuc, β-Gl and MUP mRNAs were amplified as described previously (Chiu et al., 2004; Lejeune et al., 2003), and RLuc mRNA was amplified using the primers 5′-ATGACTTCGAAAGTTTATG-3′ (SEQ ID NO. 39) (sense) and 5′-TTCAGATTTGATCAACGCA-3′ (SEQ ID NO. 40) (antisense). Cellular Arf1 mRNA or Arf1 mRNA that derived from a pSport vector was amplified using the primers 5′-AACCAACGCCTGGCTCGG-3′ (SEQ ID NO. 41) (sense) and 5′-AGTCCTTCATAGAGCCCGTCG-3′ (SEQ ID NO. 42) (antisense) or 5′-GCTATTTAGGTGACACTATAGAAGGTAC-3′ (SEQ ID NO. 43) (sense) and 5′-TTCTTGTACTCCACGGTTTC-3′ (SEQ ID NO. 44) (antisense), respectively.

248. RT-PCR products were electrophoresed in 5% polyacrylamide and quantitated by PhosphorImaging (Molecular Dynamics).

249. In experiments that mapped Stau1-HA binding within the Arf1 mRNA 3' UTR, RT-PCR analysis of Arf1 mRNA was performed using One Step RT-PCR (Qiagen), the primer pair 5'-GCTATTTAGGTGACACTATAGAAGGTAC-3' (SEQ ID NO. 45) (sense) and 5'- CTCTGTCATTGCTGTCCACCACG-3' (SEQ ID NO. 46) (antisense), and ethidium bromide staining.

250. FLuc-MS2bs mRNA or FLuc(UAA→CAA)-MS2bs mRNA was amplified using the primers 5′-CAACACCCCAACATCTTCG-3′ (SEQ ID NO. 47) (sense) and 5′-CTTTCCGCCCTTCTTGGCC-3′ (SEQ ID NO. 48) (antisense). Gl-MS2bs or Gl(UAA→UAC)-MS2bs was amplified using the primers 5′-AATACGACTCACTATAGGGA-3′ (SEQ ID NO. 49) (sense), which anneals to the T7 promoter, and 5′-GATACTTGTGGGCCAGGGCA-3′ (SEQ ID NO. 50) (antisense). FLuc-Arf1 mRNA was amplified using the same T7 promoter primer (sense) and 5′-TCTAGAGGATAGAATGGCG-3′ (SEQ ID NO. 51) (antisense). PAICS mRNA was amplified using the primers 5′-AGCAGGCTGGTACCGGTCCG-3′ (SEQ ID NO. 52) (sense) and 5′-ACCAATGTTCAGTACCTCAG-3′ (SEQ ID NO. 53) (antisense).

Example 14: Far-Western Blotting

251. FLAG-Upf1 was purified from HeLa cells that had been stably transfected with pCI-neo-FLAG-UPF1 as previously described (Pal et al., 2001). GST-Upf1 was purified from E. coli using Bulk GST Purification Modules (Amersham).

252. Lysates of E. coli that were or were not induced to express 6xHis-Stau1 or GST-Stau1 using IPTG (Invitrogen) were resolved in 8% SDS-polyacrylamide and transferred to Hybond ECL nitrocellulose (Amersham). Membranes were incubated for 12 h at 4°C in blocking buffer [20 mM Hepes (pH 7.4), 50 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, 0.1% NP-40 and 2% milk], and then incubated overnight at 4°C in blocking buffer containing 10 μg of purified FLAG-Upf1 or GST-Upf1. Interacting proteins were detected using Western blotting and anti-FLAG or anti-GST antibody.

Example 15: Microarray Analysis

253. The IP of Stau1-containing RNP was performed as previously described (Duchaine et al., 2000). Constituent RNAs were purified and deemed 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 U133A 22000 human genes chips. Hybridized chips were scanned using an Agilent GeneArray scanner 2500 (Affymetrix), and scanned images were analyzed using Microarray Analysis Suite version 5.0 (Affymetrix). Notably, the Affymetrix Gene Expression Assay identifies changes that are greater than 2-fold with 98% accuracy (Wodicka et al., 1997). Changes of at least 2.5-fold were scored as Stau1-interacting transcripts.

Example 16: Identification of HeLa-cell transcripts are regulated upon Stau1 depletion

254. 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 6).

Table 6: Transcripts upregulated in human cells depleted of Stau1 in three independently performed microarray analyses

Transcript	*Fold change*	*Abbreviation*	*Probe set*

Homo sapiens 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 (fasciclin 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
Homo sapiens transcribed sequence with weak similarity to protein ref:NP_060265.1 (*H.sapiens*) hypothetical protein FLJ20378 [Homo sapiens]	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
Homo sapiens 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
Homo sapiens uncharacterized gastric protein ZA43P mRNA partial cds	3.43		232696_at
Homo sapiens similar to KIAA0563-related gene (LOC376854) mRNA	3.42		1562921_at
hypothetical protein FLJ20035	3.40	FLJ20035	218986_s_at
Homo sapiens 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
Homo sapiens 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	ARHGDIB	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
Homo sapiens 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
Homo sapiens BIC noncoding mRNA complete sequence	2.69		229437_at
transducin-like enhancer of split 4 (E(sp1) homolog *Drosophila*)	2.68	TLE4	235765_at
dapper homolog 1 antagonist of beta-catenin (xenopus)	2.67	DACT1	219179_at
chromosome 9 open reading frame 39	2.64	C9orf39	220095_at
Homo sapiens transcribed sequences	2.61		229242_at
v-jun sarcoma virus 17 oncogene homolog (avian)	2.58	JUN	201466_s_at
integrin alpha 4 (antigen CD49D alpha 4 subunit of VLA-4 receptor)	2.56	ITGA4	205885_s_at
Homo sapiens cDNA FLJ40697 fis clone THYMU2025406	2.53		235203_at
Homo sapiens transcribed sequences	2.53		236817_at
tenascin C (hexabrachion)	2.52	TNC	201645_at
hypothetical protein FLJ13621	2.50	FLJ13621	207286_at
flavoprotein oxidoreductase MICAL2	2.47	MICAL2	212473_s_at
Homo sapiens cDNA FLJ46457 fis clone THYMU3020856	2.44		225007_at
thymosin beta identified in neuroblastoma cells	2.44	TMSNB	205347_s_at
prostaglandin E receptor 4 (subtype EP4)	2.42	PTGER4	204897_at
chromosome 14 open reading frame 128	2.41	C14orf128	228889_at
ganglioside-induced differentiation-associated protein 1	2.41	GDAP1	226269_at
zinc finger protein 36 C3H type-like 1	2.38	ZFP36L1	211965_at
uracil-DNA glycosylase	2.37	UNG	202330_s_at
hypothetical protein MGC27277	2.37	MGC27277	242283_at
Homo sapiens cDNA FLJ10158 fis clone HEMBA1003463.	2.35		232125_at
CDK5 regulatory subunit associated protein 1-like 1	2.34	CDKAL1	214877_at
Homo sapiens transcribed sequence with moderate similarity to protein ref:NP_071431.1 (*H.sapiens*) 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
Homo sapiens 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
Homo sapiens 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	TGFB1I1	209651_at
ets variant gene 1	2.15	ETV1	221911_at

Rho GTPase activating protein 19	2.14	ARHGAP19	212738_at
Homo sapiens 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
Homo sapiens transcribed sequences	2.09		244091_at
spinal cord-derived growth factor-B	2.09	SCDGF-B	219304_s_at
Homo sapiens cDNA FLJ37290 fis clone BRAMY2014469.	2.09		231026_at
histone deacetylase 8	2.07	HDAC8	223908_at
	2.06		1567079_at
Homo sapiens transcribed sequence with weak similarity to protein pir:I38588 (*H.sapiens*) I38588 reverse transcriptase homolog - human retrotransposon L1	2.06		235302_at
Homo sapiens 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
Homo sapiens cDNA FLJ33441 fis clone BRACE2021932.	2.04		1556081_at
hypothetical protein DKFZp761K1423	2.04	DKFZp761K 1423	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 (*Drosophila*)	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

Furthermore, 115 transcripts, or 1% of the HeLa-cell transcriptome that was analyzed, were downregulated at least 2-fold in all three transfections (Table 7).

Table 7 Transcripts downregulated in human cells depleted of Staul in three independently performed microarray analyses

Transcript	*Fold change*	*Abbreviation*	*Probe set*
transmembrane protein 9	0.50	TMEM9	222988_s_at
MAX dimerization protein 1	0.50	MAD	228846_at
ecotropic viral integration site 5	0.50	EVI5	209717_at
NEDD8-conjugating enzyme	0.50	NCE2	225783_at
ATPase H+ transporting lysosomal 9kDa V0 subunit e	0.49	ATP6V0E	236527_at
RAP2A member of RAS oncogene family	0.49	RAP2A	225585_at
T-cell leukemia translocation altered gene	0.49	TCTA	203054_s_at
ubiquitin-conjugating enzyme E2R 2	0.49	UBE2R2	226954_at
hypothetical protein FLJ30794	0.49	FLJ30794	238029_s_at
kelch-like 8 (*Drosophila*)	0.49	KLHL8	242648_at
junction-mediating and regulatory protein	0.49	JMY	241985_at
tripartite motif-containing 37	0.49	TRIM37	213009_s_at
tubulin-tyrosine ligase	0.48	TTL	224896_s_at
cystathionase (cystathionine gamma-lyase)	0.48	CTH	217127_at
LSM1 homolog U6 small nuclear RNA associated (S. cerevisiae)	0.48	LSM1	203534_at
protein kinase H11	0.48	H11	221667_s_at
N-myc downstream regulated gene 1	0.48	NDRG1	200632_s_at

Homo sapiens mRNA	0.48		230251_at
transmembrane 4 superfamily member 1	0.48	TM4SF1	215034_s_at
guanine nucleotide binding protein (G protein) alpha inhibiting activity polypeptide 1	0.48	GNAI1	227692_at
v-raf murine sarcoma 3611 viral oncogene homolog 1	0.48	ARAF1	230652_at
isocitrate dehydrogenase 1 (NADP+) soluble	0.48	IDH1	201193_at
solute carrier family 39 (zinc transporter) member 9	0.48	SLC39A9	222445_at
tumor necrosis factor receptor superfamily member 11b (osteoprotegerin)	0.47	TNFRSF11B	204932_at
insulin induced gene 1	0.47	INSIG1	201626_at
ALL1 fused gene from 5q31	0.47	AF5Q31	243487_at
solute carrier organic anion transporter family member 3A1	0.47	SLCO3A1	210542_s_at
hypothetical protein LOC154807	0.47	LOC154807	1553679_s_at
Homo sapiens cDNA clone IMAGE:4304686 partial cds	0.47		1559007_s_at
Homo sapiens transcribed sequence with moderate similarity to protein pdb:1LBG (*E. coli*) B Chain B Lactose Operon Repressor Bound To 21-Base Pair Symmetric Operator Dna Alpha Carbons Only	0.47		230048_at
SAR1a gene homolog 2 (S. cerevisiae)	0.47	SARA2	1554482_a_at
putative nucleic acid binding protein RY-1	0.47	RY1	212440_at
TATA element modulatory factor 1	0.47	TMF1	227685_at
hydroxysteroid (17-beta) dehydrogenase 12	0.46	HSD17B12	217869_at
disabled homolog 2 mitogen-responsive phosphoprotein (*Drosophila*)	0.46	DAB2	201280_s_at
ATP citrate lyase	0.46	ACLY	210337_s_at
chondroitin beta14 N-acetylgalactosaminyltransferase	0.46	ChGn	219049_at
chromosome 9 open reading frame 12	0.46	C9orf12	219092_s_at
hypothetical protein DKFZp762K222	0.46	DKFZp762K222	231969_at
inhibitor of DNA binding 2 dominant negative helix-loop-helix protein	0.46	ID2	201565_s_at
dual specificity phosphatase 1	0.46	DUSP1	201041_s_at
Homo sapiens transcribed sequences	0.45		238577_s_at
transforming growth factor beta receptor III (betaglycan 300kDa)	0.45	TGFBR3	226625_at
GABA(A) receptors associated protein like 3	0.45	GABARAPL3	211458_s_at
nuclear receptor subfamily 4 group A member 2	0.45	NR4A2	204622_x_at
Homo sapiens cDNA FLJ38505 fis clone HCHON2000226.	0.45		227443_at
synaptosomal-associated protein 23kDa	0.45	SNAP23	209131_s_at
RWD domain containing 3	0.44	RWDD3	205087_at
retinoic acid early transcript 1E	0.44	RAET1E	1552777_a_at
polymerase (RNA) II (DNA directed) polypeptide L 7.6kDa	0.44	POLR2L	202586_at
kelch-like 2 Mayven (*Drosophila*)	0.44	KLHL2	219157_at
solute carrier family 18 (vesicular monoamine) member 2	0.44	SLC18A2	230416_at
sideroflexin 1	0.44	SFXN1	230069_at
KIAA0256 gene product	0.43	KIAA0256	212450_at
	0.43		227278_at
GABA(A) receptor-associated protein like 1	0.43	GABARAPL1	208868_s_at
solute carrier family 11 (proton-coupled divalent metal ion transporters) member 2	0.43	SLC11A2	203124_s_at
sphingomyelin phosphodiesterase acid-like 3A	0.43	SMPDL3A	213624_at
neuronal pentraxin I	0.43	NPTX1	204684_at
fucosyltransferase 11 (alpha (13) fucosyltransferase)	0.43	FUT11	226348_at
4-hydroxyphenylpyruvate dioxygenase	0.43	HPD	206024_at

diphtheria toxin receptor (heparin-binding epidermal growth factor-like growth factor)	0.43	DTR	203821_at
hypothetical protein DKFZp762C1112	0.43	DKFZp762C1112	225974_at
sorting nexin 13	0.43	SNX13	227031_at
fibronectin leucine rich transmembrane protein 2	0.42	FLRT2	204359_at
START domain containing 7	0.42	STARD7	200028_s_at
ubiquitin specific protease 31	0.42	USP31	229812_at
Homo sapiens cDNA FLJ37284 fis clone BRAMY2013590.	0.42		225728_at
protein tyrosine phosphatase type IVA member 1	0.42	PTP4A1	200732_s_at
glutamate-cysteine ligase modifier subunit	0.42	GCLM	234986_at
guanine nucleotide binding protein (G protein) alpha activating activity polypeptide olfactory type	0.42	GNAL	218178_s_at
chromosome 13 open reading frame 1	0.42	C13orf1	230151_at
heat shock 70kDa protein 9B (mortalin-2)	0.42	HSPA9B	200690_at
FK506 binding protein 14 22 kDa	0.42	FKBP14	235311_at
adducin 1 (alpha) /// adducin 1 (alpha)	0.42	ADD1	208030_s_at
hypothetical protein FLJ20151	0.41	FLJ20151	214164_x_at
sestrin 3	0.41	SESN3	225123_at
Homo sapiens transcribed sequence with strong similarity to protein ref:NP_078816.1 (H.sapiens) hypothetical protein FLJ20917 [Homo sapiens]	0.41		230728_at
Arg/Abl-interacting protein ArgBP2	0.41	ARGBP2	204288_s_at
adaptor-related protein complex 1 gamma 1 subunit	0.41	AP1G1	215867_x_at
cyclin C	0.41	CCNC	201955_at
collagen type XXV alpha 1	0.41	COL25A1	1555253_at
hypothetical protein BC013767	0.40	LOC114990	225867_at
KIAA0877 protein	0.40	KIAA0877	212792_at
HSPC163 protein	0.40	HSPC163	228437_at
hypothetical protein FLJ14213	0.39	FLJ14213	219383_at
Homo sapiens mRNA	0.39		227628_at
family with sequence similarity 20 member B	0.39	FAM20B	202916_s_at
likely ortholog of mouse hypoxia induced gene 1	0.39	HIG1	221896_s_at
nuclear receptor subfamily 4 group A member 3	0.38	NR4A3	207978_s_at
aldo-keto reductase family 1 member C1 (dihydrodiol dehydrogenase 1	0.38	AKR1C1	1555854_at
hypothetical protein FLJ90492	0.37	FLJ90492	226604_at
low density lipoprotein receptor-related protein 11	0.37	LRP11	225060_at
junctophilin 1	0.37	JPH1	229139_at
calumenin	0.37	CALU	200756_x_at
solute carrier family 2 (facilitated glucose transporter) member 13	0.37	SLC2A13	1552695_a_at
Homo sapiens Similar to LOC166075 clone IMAGE:5173621 mRNA partial cds	0.36		236738_at
delta sleep inducing peptide immunoreactor	0.36	DSIPI	208763_s_at
sperm associated antigen 1	0.36	SPAG1	210117_at
carbonic anhydrase XII	0.35	CA12	210735_s_at
inositol 145-triphosphate receptor type 1	0.34	ITPR1	216944_s_at
uronyl-2-sulfotransferase	0.34	UST	205139_s_at
stanniocalcin 2	0.34	STC2	203438_at
clusterin (complement lysis inhibitor SP-4040 sulfated glycoprotein 2 testosterone-repressed prostate message 2 apolipoprotein J)	0.34	CLU	222043_at
adaptor-related protein complex 1 sigma 3 subunit	0.31	AP1S3	237159_x_at
Homo sapiens cDNA FLJ20769 fis clone COL06674	0.31		232060_at
ADP-ribosylation-like factor 6-interacting protein 6	0.31	MGC33864	225711_at

abhydrolase domain containing 3	0.31	ABHD3	213017_at
pyruvate dehydrogenase kinase isoenzyme 4	0.31	PDK4	205960_at
tumor necrosis factor receptor superfamily member 10b	0.30	TNFRSF10B	209295_at
aspartate beta-hydroxylase	0.30	ASPH	242037_at
Homo sapiens mRNA	0.29		228141_at
cell cycle progression 8 protein	0.28	CPR8	221156_x_at
thioredoxin interacting protein	0.26	TXNIP	201008_s_at
staufen RNA binding protein (*Drosophila*)	0.24	STAU1	211505_s_at

255. 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 8).

Table 8 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'	SEQ ID NO: 99	5'-TCA GCC CCC GAC GGT CTC TC-3'	SEQ ID NO: 100
SERPINE1	5'-ACC GCC AAT CGC AAG GCA CC-3'	SEQ ID NO: 101	5'-GCT GAT CTC ATC CTT GTT CC-3'	SEQ ID NO: 102
IL7R	5'-AAG TGG CTA TGC TCA AAA TG-3'	SEQ ID NO: 103	5'-TTC AGG CAC TTT ACC TCC AC-3'	SEQ ID NO: 104
IF	5'-CCT TGA CCT TGG GTT TCA AC-3'	SEQ ID NO: 105	5'-ATT TGC AAT GGA AGC CTT TG-3'	SEQ ID NO: 106
GAP43	5'-TGT GCT GTA TGA GAA GAA CC-3'	SEQ ID NO: 107	5'-GCT TCA TCC TTC TTA TTA GC-3'	SEQ ID NO: 108
CKMT1	5'-GAC TGG CCA GAT GCT CGT GG-3'	SEQ ID NO: 109	5'-ATC TTT GGG AAG CGG CTA TC-3'	SEQ ID NO: 110
STMN3	5'-CCA TGG CCA GCA CCA TTT CC-3'	SEQ ID NO: 111	5'-ACC TCG GCC GCG TGC AGC TC-3'	SEQ ID NO: 112
TAGLN	5'-TCC TTC CTG CGA GCC CTG AG-3'	SEQ ID NO: 113	5'-GCA CTG CTG CCA TGT CTT TG-3'	SEQ ID NO: 114
OASL	5'-TGC AAT CAT TGA GGA TTG TG-3'	SEQ ID NO: 115	5'-CAC TGT CAA GTG GAT GTC TC-3'	SEQ ID NO: 116
GDI1	5'-GAC AGA GAC GTG AAG CAC TG-3'	SEQ ID NO: 117	5'-CCA TAA ATG TTG CTT TAT CC-3'	SEQ ID NO: 118
CXCL1	5'-CCT GGT AGC CGC TGG CCG GC-3'	SEQ ID NO: 119	5'-CTT CTG GTC AGT TGG ATT TG-3'	SEQ ID NO: 120
DCP2	5'-GAT TTA TGT TGT TGT AGT TG-3'	SEQ ID NO: 121	5'-CCA AGC AGC CAA TTT TAT TG-3'	SEQ ID NO: 122
TMSL8	5'-ACA GCC TTT CAC GAG TCT TC-3'	SEQ ID NO: 123	5'-CTG CTG TTG GGA GGC GAT CC-3'	SEQ ID NO: 124
PSMB9	5'-GCG GGA GAA GTC CAC ACC GG-3'	SEQ ID NO: 125	5'-AGG CTG TCG AGT CAG CAT TC-3'	SEQ ID NO: 126
GDAP1	5'-AGT TAA CTG TGG ACT CCA TG-3'	SEQ ID NO: 127	5'-ACT TTC TCC AAC TCA TCA AG-3'	SEQ ID NO: 128
STC2	5'-CCT GTC CCT GCA GAA TAC AG-3'	SEQ ID NO: 129	5'-GTT CAC GAG GTC CAC GTA GG-3'	SEQ ID NO: 130
DUSP	5'-GCA AGT CTT CTT CCT CAA AG-3'	SEQ ID NO: 131	5'-TTC CTC CAG CAT TCT TGA TG-3'	SEQ ID NO: 132
GABARAPL1	5'-AAA TAT CCG GAC AGG GTC CC-3'	SEQ ID NO: 133	5'-TAG TCT TCC TCA TGA TTG TC-3'	SEQ ID NO: 134
TXNIP	5'-TCT GGA AGA CCA GCC AAC AG-3'	SEQ ID NO: 135	5'-TCA GCA TGG ATG GAA ATC TC-3'	SEQ ID NO: 136
TNFRSF10B	5'-TCG CCG CGG TCC TGC TGT TG-3'	SEQ ID NO: 137	5'-TGA CCA TCC CTC TGG GAC AC-3'	SEQ ID NO: 138

CCPG1	5'-GGT CTG GAC TGA TGA AAA TC-3'	SEQ ID NO: 139	5'-CAC TGT GCC ATT TTG GCT GC-3'	SEQ ID NO: 140

Results demonstrated that 11 of the 12 were increased in abundance by 1.5-fold to 8.5-fold (Figure 15) and 6 of the 6 were decreased in abundance by 2-fold to 10-fold (Figure 16) upon Staul depletion. Therefore, the microarray results can generally be viewed as a reliable assessment of changes in transcript abundance upon Staul depletion.

256. Transcripts that were upregulated upon Staul depletion and, thus, represent possible SMD targets encode proteins that are involved in signal transduction, cell proliferation or both (Table 9).

Table 9 Selected examples of transcripts upregulated in human cells depleted of Staul in three independently performed microarray analyses

V. Cell motility, cell adhesion, extracellular matrix

actin alpha 2 smooth muscle aorta
chromosome 14 open reading frame 141
fibronectin 1
filamin-binding LIM protein-1
insulin-like growth factor binding protein 5
integrin alpha 4 (antigen CD49D alpha 4 subunit of VLA-4 receptor)
integrin beta 3 (platelet glycoprotein IIIa antigen CD61)
osteoblast specific factor 2 (fasciclin I-like)
protein tyrosine phosphatase receptor type F
tenascin C (hexabrachion)
thrombospondin 1
transforming growth factor beta 1 induced transcript 1

VI. Structural molecule activity, cytoskeleton, cytoskeleton organization and biogenesis

actin alpha 2 smooth muscle aorta
actinin alpha 1
chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity alpha)
filamin-binding LIM protein-1
Rho GDP dissociation inhibitor (GDI) beta
thymosin beta identified in neuroblastoma cells
tissue factor pathway inhibitor 2

VII. Regulation of cell growth, cell cycle

chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity alpha)
chromosome 14 open reading frame 141
cyclin-dependent kinase 5 regulatory subunit 1 (p35)
cyclin-E binding protein 1
dual specificity phosphatase 6
ets variant gene 1
growth associated protein 43
histone deacetylase 8
signaling lymphocytic activation molecule family member 1
spinal cord-derived growth factor-B
suppressor of cytokine signaling 2
tumor protein p53 (Li-Fraumeni syndrome)
v-jun sarcoma virus 17 oncogene homolog (avian)

VIII. Apoptosis

catenin (cadherin-associated protein) alpha-like 1
TIA1 cytotoxic granule-associated RNA binding protein
tumor protein p53 (Li-Fraumeni syndrome)

IX. Immune response

2'-5'-oligoadenylate synthetase-like
chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity alpha)
fibronectin 1
guanylate binding protein 1 interferon-inducible 67kDa
interferon alpha-inducible protein (clone IFI-15K)
interferon-induced protein with tetratricopeptide repeats 1
interferon-induced protein with tetratricopeptide repeats 2
interferon-induced protein with tetratricopeptide repeats 4
interleukin 7 receptor
prostaglandin E receptor 4 (subtype EP4)
proteasome (prosome macropain) subunit beta type 9 (large multifunctional protease 2)
Rho GDP dissociation inhibitor (GDI) beta
signaling lymphocytic activation molecule family member 1

X. Response to stress (extracelluar stimuli)

chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity alpha)
fibronectin 1
growth associated protein 43
integrin beta 3 (platelet glycoprotein IIIa antigen CD61)
interferon-induced protein 44
interleukin 7 receptor
signaling lymphocytic activation molecule family member 1
tissue factor pathway inhibitor 2

XI. Regulation of DNA recombination, DNA repair

interleukin 7 receptor
tumor protein p53 (Li-Fraumeni syndrome)
uracil-DNA glycosylase

XII. Regulation of transcription

ets variant gene 1
hairy and enhancer of split 1 (*Drosophila*)
high-mobility group box 3
leucine rich repeat (in FLII) interacting protein 1
PHD finger protein 11
transducin-like enhancer of split 4 (E(sp1) homolog *Drosophila*)
transforming growth factor beta 1 induced transcript 1
tumor protein p53 (Li-Fraumeni syndrome)
v-jun sarcoma virus 17 oncogene homolog (avian)
zinc finger protein 90 homolog (mouse)

XIII. Regulation of translation

eukaryotic translation initiation factor 5A2

XIV. Protein secretion

secretogranin II (chromogranin C)

XV. Signal cascade

2'-5'-oligoadenylate synthetase-like
chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity alpha)
chromosome 14 open reading frame 141
dihydropyrimidinase-like 3
dual specificity phosphatase 6
growth associated protein 43
guanine nucleotide binding protein (G protein) gamma 4
hypothetical protein FLJ33505
insulin-like growth factor binding protein 5
integrin alpha 4 (antigen CD49D alpha 4 subunit of VLA-4 receptor)
integrin beta 3 (platelet glycoprotein IIIa antigen CD61)
interleukin 7 receptor
prostaglandin E receptor 4 (subtype EP4)
protein tyrosine phosphatase receptor type F
protein tyrosine phosphatase receptor type O
Rho GDP dissociation inhibitor (GDI) beta
signaling lymphocytic activation molecule family member 1
spinal cord-derived growth factor-B
stathmin-like 3
suppressor of cytokine signaling 2
tenascin C (hexabrachion)
thrombospondin 1
transducin-like enhancer of split 4 (E(sp1) homolog *Drosophila*)
transforming growth factor beta 1 induced transcript 1
type I transmembrane receptor (seizure-related protein)

XVI. Complement and coagulation cascades

I factor (complement)
serine (or cysteine) proteinase inhibitor clade E
thrombospondin 1
tissue factor pathway inhibitor 2

XVII. Cellular metabolism

aldehyde dehydrogenase 1 family member A3
CGI-72 protein
cyclin-E binding protein 1
dihydropyrimidinase-like 3
enoyl-Coenzyme A hydratase/3-hydroxyacyl Coenzyme A dehydrogenase
hypothetical protein FLJ25348
protein tyrosine phosphatase receptor type O
selenocysteine lyase
uracil-DNA glycosylase

XVIII. Development, Morphogenesis, Organogenesis

actin alpha 2 smooth muscle aorta
chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity alpha)
cyclin-dependent kinase 5 regulatory subunit 1 (p35)
dihydropyrimidinase-like 3
filamin-binding LIM protein-1
growth associated protein 43
hairy and enhancer of split 1 (*Drosophila*)
high-mobility group box 3
osteoblast specific factor 2 (fasciclin I-like)

Rho GDP dissociation inhibitor (GDI) beta
stathmin-like 3
suppressor of cytokine signaling 2
thrombospondin 1
transducin-like enhancer of split 4 (E(sp1) homolog *Drosophila*)
transgelin
tumor protein p53 (Li-Fraumeni syndrome)

XIX. **Organelle organization and biogenesis**

thymosin beta identified in neuroblastoma cells

XX. Oxidoreductase activity

fibronectin 1

XXI. Proteasome, ubiquitin cycle

cyclin-E binding protein 1
hypothetical protein FLJ20637
proteasome (prosome macropain) subunit beta type 9 (large multifunctional protease 2)

Other transcripts that were upregulated encode proteins that function in the immune response. Still others produce proteins that participate in cell adhesion, motility, the extracellular matrix, or other aspects of cell structure. A number encode factors that regulate transcription. Others encode proteins involved in RNA metabolism, including the TIA1 cytotoxic granule-associated RNA binding protein, which regulates the alternative splicing of pre-mRNA that encodes the human apoptotic factor Fas(Forch et al., 2002) and translationally silences mRNAs that encode inhibitors of apotosis such as tumor necrosis factor α (TNF-a)(Piecyk et al., 2000; Li et al., 2004). Another RNA metabolic protein that was upregulated upon Stau1 depletion is Dcp2, which mediates transcript decapping(Wang et al., 2002). It is worth noting, however, that the abundance of an mRNA could be upregulated upon Stau1 depletion via a mechanism that involves an alteration in its half-life, such as SMD, or via the product of another mRNA that itself is directly regulated by Stau1.

Example 17: Stau1 or Upf1 depletion increases the abundance of c-JUN, SERPINE and IL7R 3' mRNAs

257. 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 (Mendell et al., 2004; Table 10).

Table 10 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 (nexin plasminogen activator inhibitor type 1) member 1 (SERPINE1)	Bait for tissue plasminogen activator, urokinase, and protein C	3.2	3.8
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).

258. 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.

259. 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 17A, 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 17B, where each transcript is normalized to the level of SMG7 mRNA). These results indicate that each transcript is targeted for SMD.

Example 18: Stau1 binds the 3' UTR of c-JUN, SERPINE and IL7R 3' mRNAs

260. 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 (Kim et al., 2005). 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-HA_3 binding.

261. Cos cells were transfected with four test plasmids: pcFLuc-c-JUN 3'UTR, pcFLuc-SERPINE1 3'UTR, pcFLuc-IL7R 3'UTR and pcFLuc-Arf1 SBS (Figure 18A), the latter of which serves as a positive control for Stau1-HA_3 binding since it contains the minimized Stau1 binding site (SBS) from the Arf1 3'UTR (Kim et al., 2005). Cells were simultaneously transfected with two additional plasmids: phStau1-HA_3, which produces Stau1-HA_3, and pmCMV-MUP, which serves as a negative control for Stau1-HA_3 binding. In cells producing Stau1-HA_3 (Figure 18B), anti-HA immunopurified FLuc-Arf1 SBS mRNA as well as FLuc-c-JUN 3'UTR, FLuc-SERPINE1 3'UTR and FLuc-IL7R 3'UTR mRNAs, but not MUP mRNA (Figure 18C). In contrast, rat (r) IgG, which controls for nonspecific immunopurification, failed to immunopurify any of the transcripts (Figure 18C). Furthermore, anti-HA failed to immunopurify FLuc mRNA that harbors the FLuc 3'UTR. Therefore, the 3'UTRs of c-JUN, SERPINE1 and IL7R mRNAs bind Stau1-HA_3, as do the two known physiologic targets of SMD (Kim et al., 2005). Notably, a larger fraction of FLuc-Arf1 SBS mRNA was bound by Stau1-HA_3 relative FLuc-c-JUN 3'UTR, FLuc-SERPINE1 3'UTR or FLuc-IL7R 3'UTR mRNA (Figure 18C), which is consistent with detection of Arf1 mRNA but not c-JUN, SERPINE1 or IL7R mRNA in the earlier microarray analyses of transcripts that bind Stau1-HA_3.

Example 19: c-JUN, SERPINE1 and IL7R 3' UTRs trigger SMD

262. To determine if each 3'UTR sequence is sufficient to elicit SMD, the effect of depleting Stau1 or Upf1 on the half-life of FLuc-c-JUN 3'UTR, FLuc-SERPINE1 3'UTR, or FLuc-IL7R 3'UTR mRNA or, as a positive control, FLuc-Arf1-SBS mRNA was tested. Production of each mRNA was driven by the fos promoter (Figure 19A). This promoter is transiently inducible upon the addition of serum to serum-deprived mouse L cells and therefore provides a way to analyze mRNA half-life (Kim et al., 2005; Lejeune et al., 2003). L cells were transfected with mouse (m)Stau1 siRNA, mUpf1 siRNA or a nonspecific Control siRNA (Kim et al., 2005) and, two days later, with the four pfos-FLuc test plasmids and the phCMV-MUP reference plasmid. The reference plasmid controls for variations in transfection

efficiencies and RNA recovery. One day later, serum was added, and protein and RNA were purified from, respectively, cytoplasmic and nuclear fractions after 0, 30, 45 and 60 minutes. Under conditions where either mStau1 or mUpf1 was depleted (Figure 19B), the half-life of each fos-FLuc mRNA was increased in each of two or three independently performed experiments (Figure 19C). Therefore, Stau1 and Upf1 together with the 3'UTR of c-JUN, SERPINE1 or IL7R mRNAs mediate mRNA decay, indicating that c-JUN, SERPINE1 and IL7R mRNAs are bone fide SMD targets.

Example 20: GAP43 mRNA is an SMD target

263. The finding that SMD is conferred by 191 nucleotides of the c-JUN 3'UTR, 151 of which constitute the c-JUN class III ARE, together with microarray data indicating the class III ARE-containing mRNA for GAP43 is also upregulated when Stau1 is depleted (Table 9; Figure 15), led to testing to determine if GAP43 mRNA is another SMD target.

264. Using RNA from samples analyzed in Figure 17, depleting Stau1 or Upf1 was found to increase the cellular abundance of GAP43 mRNA 3.8-fold to 4.6-fold whereas depleting Upf2 was of no appreciable consequence to GAP43 mRNA abundance. (Figure 20A).

265. In cells producing Stau1-HA_3, anti-HA immunopurified FLuc-GAP43 3' UTR mRNA (Figure 20B, right), in which the FLuc 3' UTR had been replaced with the nucleotides 1-423 of the 3' UTR of GAP43 mRNA and, as a positive control, FLuc-Arf1 SBS mRNA (Figure 20B, left). In contrast, rIgG immunopurified neither mRNA.

266. Finally, in experiments that utilized pfos-FLuc-GAP43 3' UTR (Figure 20C, upper left), depleting L cells of mStau1 or mUpf1 (Figure 20C, upper right) increased the half-life of fos-FLuc-GAP43 3' UTR mRNA (Figure 20C, lower left and right). Therefore, GAP43 mRNA is another SMD target.

Example 21: RNA motifs that bind Stau1

267. The best characterized Staufen binding site exists within Drosophila bicoid mRNA (Ferrandon et al., 1994). Linker scanning mutations that disrupted the interaction of Staufen with this mRNA mapped to three noncontiguous regions: 148 nucleotides of stem III, 89 nucleotides of the distal region of stem IV, and 88 nucleotides of the distal region of stem V (Ferrandon et al., 1994). Given the structural complexity of human Staufen binding site(s), it may be difficult to identify human Stau1 binding sites exclusively from our existing mRNA immunopurification and half-life data. Additionally, while the double-stranded RNA binding domain 3 of Drosophila Staufen has been proposed to mediate direct binding of the protein to

bicoid and oskar mRNAs (Micklem et al., 2000; Ramos et al., 2000), it is uncertain whether or not binding specificity can be influenced by other proteins. To complicate matters further, sequence disparities between Drosophila Staufen and human Stau1 likely confer differences in RNA binding specificity so that data pertaining to Drosophila Staufen may not be applicable to human Stau1. Therefore, we aimed to more thoroughly characterize each of the 3' UTR sequences that were shown to bind Stau1 (Figures 18 and 20) and target an mRNA for SMD (Figures 19 and 20) to better define RNA sequences that bind human Stau1.

268. It is very likely that Arf1, c-JUN, SERPINE1, IL7R and GAP43 mRNAs will not be the only transcripts among those upregulated upon Stau1 depletion that are targeted for SMD. For example, CYR61 mRNA, which encodes the cysteine-rich angiogenic inducer 61, was upregulated upon Stau1 depletion in only two of our three microarray analyses and, thus, fell below the stringent criteria as a candidate target for SMD. However, this mRNA may very well be targeted for SMD since it was also among the transcripts upregulated upon Upf1 depletion (Forch et al., 2002). Furthermore, 20 transcripts (Table 11) that were upregulated upon Stau1 depletion in all three microarray analyses were also present in either two (10 transcripts) or all three (10 transcripts) new microarray analyses that assayed for Stau1-HA_3 binding (see Kim et al., 2005 for methodology).

Table 11. Transcripts upregulated in human cells depleted of Stau1 in three independently performed microarray analyses and that bind Stau1-HA_3 in three (white) or two (gray) independently performed microarray analyses

Transcript	Relative increase (microarray value)		Accession
	Stau1 depletion	Anti–HA IP	
Homo sapiens cDNA FLJ40697 fis clone THYMU2025406	2.5	16.6	AA398756
Homo sapiens transcribed sequence with moderate similarity to protein ref:NP_071431.1 (*H.sapiens*) cytokine receptor-like factor 2	2.3	9.2	AA824321
Interferon-induced protein with tetratricopeptide repeats 4 (IFIT4)	4.1	7.2	AI075407
Stathmin-like 3 (STMN3)	5.5	6.4	AL353715
Interferon alpha-inducible protein (clone IFI-15K) (G1P2)	3.2	4.1	NM_005101
Cyclin-dependent kinase 5 regulatory subunit 1 (p35) (CDK5R1)	2.1	3.8	AL567411
Decapping enzyme hDcp2 (DCP2)	2.7	2.6	AV715578
KIAA0143 protein (KIAA0143)	2.0	2.4	AW470003
Cyclin-E binding protein 1 (CEB1)	3.6	2.3	NM_016323
Rho GTPase activating protein 19 (ARHGAP19)	2.1	2.0	U79256
Guanine nucleotide binding protein (G protein) gamma 4 (GNG4)	4.8	4.3	NM_004485
Rho GTPase activating protein 19 (ARHGAP19)	2.1	2.1	AV717623
Homo sapiens cDNA FLJ37290 fis clone BRAMY2014469	2.1	7.4	AW451135
Epiplakin 1 (EPPK1)	2.3	3.9	AL137725
Fibronectin 1 (FN1)	4.7	3.3	X02761
KIAA0931 protein (KIAA0931)	2.1	3.0	AB023148
Homo sapiens similar to KIAA0563-related gene (LOC376854) mRNA	3.4	2.6	BC040700
Likely ortholog of mouse Sds3 (SDS3)	2.1	2.6	AK026749
Thioredoxin reductase 3 (TXNRD3)	2.0	2.4	AI247566

KIAA0143 protein (KIAA0143)	2.3	2.3	AA805651

Nevertheless, until an mRNA is shown to (i) bind Staul, (ii) manifest a longer half-life upon depletion of Staul and (iii) manifest a longer half-life upon depletion of Upf1, it cannot be considered to be a bone fide SMD target.

Example 22: Plasmid constructions

269. To construct pcFLuc harboring different 3'UTRs, pcFLuc-8bs (Kim et al., 2005) was digested with XbaI and ligated to one of four PCR-amplified fragments that contained the (i) c-JUN ARE III plus 45 nucleotides that had been digested with XbaI, (ii) SERPINE1 3'UTR without the normal termination codon that had been digested with XbaI, (iii) IL7R without the normal termination codon that had been digested with NheI or (iv) GAP43 3'UTR without the normal termination codon that had been digested with XbaI . The c-JUN ARE fragment was amplified using the Human HeLa BD™ Marathon-Ready cDNA (BD Biosciences) and two primers: 5'-CGCTCTAGAGTGAGAACTCTTTCTGGCCTGCCTTCGTTAAC-3' (SEQ ID NO: 63) (sense) and 5'-CGCTCTAGATTACAAATGGTAAACTCAGAGTGCTCC-3' (SEQ ID NO: 64) (antisense), where underlined nucleotides specify the XbaI site. The SERPINE1 3'UTR fragment was amplified using pCMV6-XL5-SERPINE1 (Origene Technologies) and two primers: 5'-CGCTCTAGAGTGACCCTGGGGAAAGACGCCTTCATCTG-3' (SEQ ID NO: 65) (sense) and 5'-CGCTCTAGAGCTTCTATTAGATTACATTCATTTCAC-3' (SEQ ID NO: 66) (antisense), where underlined nucleotides specify the XbaI site. The IL7R 3'UTR fragment was amplified using Human HeLa BD™ Marathon-Ready cDNA and two primers: 5'-CGCGCTAGCGTGAAGTGTAAGAAACCCAGACTGAAC-3' (SEQ ID NO: 67) (sense) and 5'-CGCGCTAGCTTTTTTTCCTCTCATGCTCTCTTCCTGC-3' (SEQ ID NO: 68) (antisense), where underlined nucleotides specify the NheI site. The GAP43 fragment was amplified using HeLa-cell total cDNA prepared from Staul siRNA-treated cells and two primers: 5'-CGCTCTAGAGTGAACTCTAAGAAATGGCTTTCCACATC-3' (sense) (SEQ ID NO: 141) and 5'-CGCTCTAGAGTGAGAATTCACTCGATATTTTGGACTCCTCAG-3' (antisense) (SEQ ID NO: 142), where underlined nucleotides specify the XbaI site.

270. To construct pfos-FLuc harboring different 3'UTRs, pfos-Gl (Kim et al., 2005) was digested with EcoRI and NcoI and ligated to one of three PCR-amplified

fragments that had been digested with EcoRI and NcoI. Each PCR fragment was amplified using the pcFLuc-c-JUN 3'UTR, pcFLuc-SERPINE1 3'UTR, or pcFLuc-IL7R 3'UTR and two primers: 5'-CATGCCATGGAAGACGCCAAAAACATAAAGAAAGGC-3' (SEQ ID NO: 69) (sense) and 5'-CGGAATTCAGGCTGATCAGCGAGCTCTAGCATTTAGG-3' (SEQ ID NO: 70) (antisense), where underlined nucleotides specify the NcoI and EcoRI site, respectively.

271. To construct pSport-Arf1-SBS derivatives harboring Δ(250-300), Δ(200-300), Δ(150-300), Δ(100-300) or Δ(50-300), pSport was digested with XbaI and HpaI and ligated to one of five PCR-amplified fragments that had been digested with XbaI. Each PCR fragment was amplified using pSport-Arf1-SBS, the common sense primer 5'-GCTATTTAGGTGACACTATAGAAGGTAC-3' (SEQ ID NO: 143) and the specific antisense primer: 5'-CTTTTTACAATAAAAAAAGCTGAGTAATAT-3' (SEQ ID NO: 144), 5'-TGCCTCATTGGAAACAAAAACTATTTACAT-3' (SEQ ID NO: 145), 5'-AGGCTGCGTCTGCCACATTTAC-3' (SEQ ID NO: 146), 5'-ACCACGGAGGCAGCTTCTGG-3' (SEQ ID NO: 147), or 5'-ATGAGAGTAAAGCAGAGGGCAAG-3' (SEQ ID NO: 148).

272. pSport-Arf1-SBS derivatives harboring Δ(3-300), Δ(30-79), and Δ(30-179), were generated with the following primer pairs, respectively: 5'-CATTTCGACAAACAAGCACTGTAATTATAGCTATTAG-3' (sense) (SEQ ID NO: 149) and 5'-GTTCACTTCTGGTTCCGGAGCTG-3' (antisense) (SEQ ID NO: 150), 5'-CCAGAAGCTGCCTCCGTGG-3' (sense) (SEQ ID NO: 151) and 5'-AAGAGGAGTGAGAGGGAGGG-3' (antisense) (SEQ ID NO: 152), 5'-TTTTTGTTTCCAATGAGGCAGTTTCTGGTA-3' (sense) (SEQ ID NO: 153) and 5'-AAGAGGAGTGAGAGGGAGGG-3' (antisense) (SEQ ID NO: 154). PCR amplifications were carried out using Pfu Ultra (Stratagene). PCR products were incubated with DpnI to digest the methylated template DNA, phosphorylated at the 5' ends using T4 polynucleotide kinase (Fermentas), and circularized by ligation.

273. To construct Single and Double, overlap-extension PCR was used. A 5' fragment was amplified using the common sense primer 5'-GCTATTTAGGTGACACTATAGAAGGTAC-3' (SEQ ID NO: 155) and 5'-CATAGGAGTACCACTTCCTGCCTCATTGG-3' (antisense, Single and Double, where underlined nucleotides specify mutated nucleotides) (SEQ ID NO: 156) or 5'-CCACGGAGGCAGAAAGTGGCACTCACACC-3' (antisense, Double) (SEQ ID NO:

157), and a 3' fragment was amplified using 5'-CCAATGAGGCAGGAAGTGGTACTCCTATG-3' (sense, Single and Double, where underlined nucleotides specify mutated nucleotides) (SEQ ID NO: 158) or 5'-GGTGTGAGTGCCACTTTCTGCCTCCGTGG-3' (sense, Double) (SEQ ID NO: 159) and the common antisense primer 5'-GTGCCCATGGGCCTACATCC-3' (SEQ ID NO: 160). The resulting fragments were mixed with the same sense and antisense primers that amplified, respectively, the 5' and 3' fragments to generate a joined product. PCR products were digested with XbaI, and inserted into the XbaI and HpaI sites of pSport. To construct Δ(Loop), a 5'fragment was amplified using the primer pair 5'-GCTATTTAGGTGACACTATAGAAGGTAC-3' (sense) (SEQ ID NO: 161) and 5'-AATTCTCGAGAGGCAGCTTCTGGCACTC-3' (antisense, where underlined nucleotides specify a XhoI site) (SEQ ID NO: 162) and inserted into the XbaI and XhoI sites of pSport. The 3'fragment was amplified using the primer pair 5'-AATTCTCGAGAGGCAGTTTCTGGTACTCCTATG-3' (sense, where underlined nucleotides specify a XhoI site) (SEQ ID NO: 163) and 5'-GTGCCCATGGGCCTACATCC-3' (antisense) (SEQ ID NO: 164), and inserted into the XhoI and HpaI sites of the 5'fragment-containing construct.

274. To construct deletions derivatives of pcFLuc-c-JUN 3' UTR, pcFLuc-8bs was digested with XbaI and ligated to one of three XbaI-digested PCR-amplified fragments that had been generated using pcFLuc-c-JUN 3'UTR and two primers: 5'-CGCTCTAGAGTGATTCGTTAACTGTGTATGTAC-3' (SEQ ID NO: 71) (sense) and 5'-CGCTCTAGAAAATTAAAAAATATATATATG-3' (SEQ ID NO: 72) (antisense) for pcFLuc-c-JUN 3'UTR (500-539), 5'-CGCTCTAGAGTGAGATGAAAGCTGATTACTGTC-3' (SEQ ID NO: 73) (sense) and 5'-CGCTCTAGAAACTTACAAAGGCATGAAGC-3' (SEQ ID NO: 74) (antisense) for pcFLuc-c-JUN 3'UTR (540-587), or 5'-CGCTCTAGAGTGAATTTCTTGTTTGTTTGTTTGGG-3' (SEQ ID NO: 75) (sense) and 5'-CGCTCTAGATGCTCCAAATCTCTTATTTAC-3' (SEQ ID NO: 76) (antisense) for pcFLuc-c-JUN 3'UTR (588-650), where underlined nucleotides specify the XbaI site.

275. To construct deletions derivatives of pcFLuc-c-JUN 3' UTR, pcFLuc-8bs was digested with XbaI and ligated to one of three XbaI-digested PCR-amplified fragments that had been generated using pcFLuc-c-JUN 3'UTR and two primers: 5'-CGCTCTAGAGTGATTCGTTAACTGTGTATGTAC-3' (sense) (SEQ ID NO: 165) and

5'-CGCTCTAGAAAATTAAAAAATATATATATG-3' (antisense) (SEQ ID NO: 166) for pcFLuc-c-JUN 3'UTR (500-539), 5'-CGCTCTAGAGTGAGATGAAAGCTGATTACTGTC-3' (sense) (SEQ ID NO: 167) and 5'-CGCTCTAGAAACTTACAAAGGCATGAAGC-3' (antisense) (SEQ ID NO: 168) for pcFLuc-c-JUN 3'UTR (540-587), or 5'-CGCTCTAGAGTGAATTTCTTGTTTGTTTGTTTGGG-3' (sense) (SEQ ID NO: 169) and 5'-CGCTCTAGATGCTCCAAATCTCTTATTTAC-3' (antisense) (SEQ ID NO: 170) for pcFLuc-c-JUN 3'UTR (588-650), where underlined nucleotides specify the XbaI site.

276. To construct deletion derivatives of pcFLuc-SERPINE1 3' UTR, pcFLuc-8bs was digested with XbaI and ligated to one of four XbaI-digested PCR-amplified fragments that had been generated using pcFLuc-SERPINE1 3'UTR and two primers: 5'-CGCTCTAGAGTGACCCTGGGGAAAGACGCCTTCATCTG-3' (sense) (SEQ ID NO: 171) and 5'-CGCTCTAGATGTCTCTCTCACCCACCCCCCCCTCAC-3' (antisense) (SEQ ID NO: 172) for pcFLuc-SERPINE1 3' UTR (1-1298), 5'-GCTCTAGAGTGATGACCCTGGGGAAAGACGCC-3' (sense) (SEQ ID NO: 173) and 5'-CGCTCTAGAGTTGGGCCACATGATGGGGG-3' (antisense) (SEQ ID NO: 174) for pcFLuc-SERPINE1 3'UTR (1-596), 5'-CGCTCTAGAGTGATCTCCTGGCCTGGCCATCTC-3' (sense) (SEQ ID NO: 175) and 5'-CGCTCTAGAGGTCAATTTCCATCAAGGGG-3' (antisense) (SEQ ID NO: 176) for pcFLuc- SERPINE1 3'UTR (597-1043), 5'-CGCTCTAGAGTGAATACAATTTCATCCTCCTTC-3' (sense) (SEQ ID NO: 177) and 5'-CGCTCTAGATGTCTCTCTCACCCACCCCC-3' (antisense) (SEQ ID NO: 178) for pcFLuc-SERPINE1 3'UTR (1044-1298), or 5'-CGCTCTAGAGCTTCTATTAGATTACATTCATTTCAC-3' (sense) (SEQ ID NO: 179) and 5'-CGCTCTAGAGTGAGGCAGCTCGGATTCAACTACCTTAG-3' (antisense) (SEQ ID NO: 180) for pcFLuc- SERPINE1 3'UTR (1299-1575), where underlined nucleotides specify the XbaI site.

277. Alternatively, to construct deletion derivatives of pcFLuc-SERPINE1 3' UTR, pcFLuc-8bs was digested with XbaI and ligated to one of four XbaI-digested PCR-amplified fragments that had been generated using pcFLuc-SERPINE1 3'UTR and two primers: 5'-CGCTCTAGAGTGATGACCCTGGGGAAAGACGCC-3' (SEQ ID NO: 77) (sense) and 5'-CGCTCTAGAGTTGGGCCACATGATGGGGG-3' (SEQ ID NO: 78) (antisense) for pcFLuc-SERPINE1 3'UTR (1-596), 5'-

CGCTCTAGAGTGATCTCCTGGCCTGGCCATCTC-3' (SEQ ID NO: 79) (sense) and 5'-CGCTCTAGAGGTCAATTTCCATCAAGGGG-3' (SEQ ID NO: 80) (antisense) for pcFLuc- SERPINE1 3'UTR (597-1043), 5'-CGCTCTAGAGTGAATACAATTTCATCCTCCTTC-3' (SEQ ID NO: 81) (sense) and 5'-CGCTCTAGATGTCTCTCTCACCCACCCCC-3' (SEQ ID NO: 82) (antisense) for pcFLuc-SERPINE1 3'UTR (1044-1298), or 5'-CGCTCTAGAGTGA GGCAGCTCGGATTCAACTAC-3' (SEQ ID NO: 83) (sense) and 5'-CGCTCTAGACATTTCACATCTGTGTGCAA-3' (SEQ ID NO: 84) (antisense) for pcFLuc-SERPINE1 3'UTR (1299-1575), where underlined nucleotides specify the XbaI site.

278. To construct pcFLuc harboring different regions of the GAP43 3' UTR, pcFLuc-8bs was digested with XbaI and ligated to one of three XbaI-digested PCR-amplified fragments that had been generated using pcFLuc-GAP43 3'UTR and two primers: 5'-CGCTCTAGAGTGAACTCTAAGAAATGGCTTTCCA-3' (SEQ ID NO: 85) (sense) and 5'-CGCTCTAGATGTTTGACTTGGGATCTTTCCTGC-3' (SEQ ID NO: 86) (antisense) for pcFLuc-GAP43 3'UTR (1-206), or 5'-CGCTCTAGAGTGAAAGTCAAACAGTGTGGCTTAAACA-3' (SEQ ID NO: 87) (sense) and 5'-CGCTCTAGAGAATTCACTCGATATTTTGGACTCCTC-3' (SEQ ID NO: 88) (antisense) for pcFLuc-GAP43 3'UTR (197-423), where underlined nucleotides specify the XbaI site.

279. To construct deletion derivatives of pcFLuc-IL7R 3' UTR, pcFLuc-8bs was digested with XbaI and ligated to one of two NheI-digested PCR-amplified fragments that had been generated using pcFLuc-IL7R 3'UTR and two primers: 5'-CGCGCTAGCGTGAAGTGTAAGAAACCCAGACTGAAC-3' (SEQ ID NO: 89) (sense) and 5'-CGCGCTAGCAACTCAGGAAGAGTGGTCAAAG-3' (SEQ ID NO: 90) (antisense) for pcFLuc-IL7R 3'UTR (1-165), or 5'-CGCGCTAGCGTGACAGTGGCACTCAACATGAGTC-3' (SEQ ID NO: 91) (sense) and 5'-CGCGCTAGCTTTTTTTCCTCTCATGCTCTCTTCCTGC-3' (SEQ ID NO: 92) (antisense) for pcFLuc-IL7R 3'UTR (166-339), where underlined nucleotides specify the NheI site.

Example 23: Cell culture and transfections, and protein and RNA purification

280. Human HeLa cells (2 x 10^6) were propagated in DMEM medium (GIBCO-BRL) containing 10% fetal bovine serum (GIBCO-BRL) in 60-mm dishes and, where specified, transiently transfected with plasmid DNA, in vitro-synthesized siRNA, or both as

described (Kim et al., 2005). Monkey Cos cells, which were used in experiment involving immunopurification, were similarly treated as were mouse L cells, which effectively support fos promoter induction upon the addition of serum after serum deprivation. All human and mouse siRNAs have been previously reported as has been the serum-induced transcription of fos-FLuc and the isolation of total-cell or nuclear protein and RNA (Kim et al., 2005).

Example 24: Western blotting and RT-PCR

281. Blotting (Kim et al., 2005) and the RT-PCR of SMG7 and MUP mRNAs were as described previously (Lejeune et al., 2003; Chiu et al., 2004). FLuc-c-JUN 3'UTR, FLuc-SERPINE1, FLuc-IL7R or FLuc-SBS mRNA was amplified using 5'-AATACGACTCACTATAGGGA-3' (SEQ ID NO: 93) (sense, which annealed to the T7 promoter that resided downstream of the CMV promoter) and, respectively, 5'-AGGCAGGCCAGAAAGAGTTC-3'(SEQ ID NO: 94), 5'-TGAAGGCGTCTTTCCCCAGG-3'(SEQ ID NO: 95), 5'-TCAGTCTGGGTTTCTTACAC-3'(SEQ ID NO: 96), or 5'-GCCTGGCCGCAGGCTGCGTC-3' (SEQ ID NO: 97) (antisense).

282. FLuc-c-JUN 3'UTR, FLuc-SERPINE1, FLuc-IL7R or FLuc-SBS mRNA that derived from pfos-FLuc constructs was amplified using the common sense primer 5'-AGACTGAGCCGATCCCGCGC-3' (SEQ ID NO: 98) and the corresponding antisense primer described above.

283. Primer pairs for the 21 transcripts in addition to c-JUN, SERPINE1 and IL7R mRNAs that were amplified to test the validity of microarray results are provided (Table 11).

Example 25: Microarray analyses

284. 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 (Wodicka et al., 1997). Arrays were undertaken using three independently generated RNA samples. Transcripts that showed at least a 2-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.

285. Throughout this application, various publications are referenced. The disclosures of these publications in their entireties 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.

286. 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.

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XXII. CLAIMS

What is claimed is:

1. A method of treating a disorder in a subject comprising administering to the subject a substance, wherein the substance modulates Staul-mediated mRNA decay (SMD.

2. The method of claim 1, wherein the SMD occurs in the pioneering round of translation.

3. The method of claim 1, wherein the SMD targets eIF4E-bound mRNA during steady-state mRNA translation.

4. The method of claim 1, wherein the disorder is an inherited genetic disorder.

5. The method of claim 4, 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, 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, lysomal enzymes disorder, steroid metabolism disorder, purine metabolism disorder, pyrimidine metabolism disorder, metal metabolism disorder, porphyrin metabolism disorder, and heme metabolism disorder.

6. The method of claim 1, wherein the disorder is an acquired disorder.

7. The method of claim 1, wherein the disorder is dementia.

8. The method of claim 6, wherein the acquired disorder is a cancer.

9. The method of claim 8, wherein the cancer is selected from the group consisting of lymphoma, B cell lymphoma, T cell lymphoma, leukemia, carcinoma, sarcoma, glioma, blastoma, neuroblastoma, plasmacytoma, 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.

10. The method of claim 1, wherein the subject is a mammal.

11. The method of claim 1, wherein the modulation is a decrease in Staul-mediated mRNA decay.

12. The method of claim 1, wherein the modulation is an increase in Staul-mediated mRNA decay.

13. The method of claim 1, wherein the substance is Staul or a complex comprising Staul and Upf1.

14. The method of claim 1, wherein the substance binds Upf1 or Staul.

15. The method of claim 1, wherein the substance is a functional nucleic acid, siRNA, peptide, protein, antibody, or small molecule.

16. A method of treating a Staul-mediated mRNA decay related disorder in a subject comprising administering to the subject Staul or a complex comprising Staul and Upf1.

17. A method of screening for a substance that modulates Staul-mediated mRNA decay (SMD) comprising

a) incubating the substance to be screened with a Staul mRNA decay complex, and

b) assaying for a change in SMD, an increase or decrease in SMD activity indicating a modulating substance.

18. The method of claim 17, wherein the complex comprises Upf1 and Staul.

19. The method of claim 17, wherein the complex one of Upf1 and Staul.

20. A method of screening for a substance that modulates Staul-mediated mRNA decay (SMD) comprising

a) incubating the substance with a stably transfected cell comprising a reporter gene with a nonsense-mutation and Staul, and

b) 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 substance indicates a substance that modulates SMD activity.

21. A method of screening for a substance that inhibits Staul-mediated mRNA decay (SMD) comprising

a) incubating the substance with Upf1 and Stau1 forming a substance-Upf1-Stau1 mixture, and

b) assaying the amount of Upf1-Stau1complex present in the mixture, a decrease in the amount of Upf1-Stau1complex relative to the amount of Upf1-Stau1complex in the absence of the substance indicating the substance inhibits SMD.

22. The method of claim 21, wherein the Upf1 has at least 80%, 85%, 90%, or 95% identity to the sequence set forth in SEQ ID NO: 3, or an SMD-active fragment thereof.

23. The method of claim 21, wherein the Stau1 has at least 80%, 85%, 90%, or 95% identity to the sequence set forth in SEQ ID NO: 1, or an SMD-active fragment thereof.

24. A method of screening for a substance that promotes Stau1-mediated mRNA decay (SMD) comprising

a) incubating the substance with Upf1 and Stau1 forming a substance- Upf1-Stau1 mixture, and

b) assaying the amount of Upf1-Stau1 complex present in the mixture, wherein a increase in the amount of Upf1-Stau1 complex relative to the amount of Upf1-Stau1 complex in the absence of the substance indicates that the substance promotes SMD.

25. The method of claim 24, wherein the Upf1 has at least 80%, 85%, 90%, or 95% identity to the sequence set forth in SEQ ID NO: 3, or a fragment thereof.

26. The method of claim 24, 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.

27. A method of screening for a substance that modulates Stau1-mediated mRNA decay (SMD) comprising,

a) administering a substance to a system, wherein the system comprises the components for SMD activity, and

b) assaying the effect of the substance 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

substance indicates the substance is a modulator.

28. A method of modulating Stau1-mediated mRNA decay (SMD) activity comprising administering a substance, wherein the substance is identified by the method of claim 27.

29. A method of making a substance that modulates Stau1-mediated mRNA decay (SMD) activity comprising admixing a substance identified by the method of claim 28 with a pharmaceutically acceptable carrier.

30. A substance made by the method of claim 29.

31. A method of making a modulator of Stau1-mediated mRNA decay (SMD) comprising,

a) administering a substance to a system, wherein the system comprises the components for SMD activity;

b) assaying the effect of the substance on the amount of SMD activity in the system;

c) selecting the substance that causes 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 substance; and

d) synthesizing the substance.

32. A substance that modulates SMD, wherein the substance is an antibody or fragment thereof that modulates SMD.

33. The substance of claim 32, wherein the antibody binds Upf1.

34. The substance of claim 32, wherein the antibody binds Stau1.

35. A substance that modulates SMD, wherein the substance is a vector comprising a nucleic acid that encodes an SMD modulator.

36. A vector comprising a nucleic acid that encodes an SMD modulator.

37. A cell comprising the vector of claim 35.

38. A substance that modulates SMD, wherein the substance is an siRNA that modulates SMD.

39. The substance of claim 38, wherein the siRNA binds Upf1.

40. The substance of claim 38, wherein the siRNA binds Stau1.

41. A method of facilitating Staul-mediated mRNA decay comprising contacting a system comprising the components for SMD with Staul.

42. A method of identifying an agent that binds a Staul binding site comprising contacting the agent to be screened with the Staul binding site.

43. The method of claim 42, wherein the Staul binding site comprises SEQ ID NO: 56.

44. A method of identifying genes modulated by the down-regulation of Staul comprising

a) incubating a substance that down-regulates SMD with a stably transfected cell comprising a reporter gene with a nonsense-mutation and Staul, and

b) assaying the amount of mRNA present for a gene in a microarray, wherein a increase or decrease in the amount of mRNA relative to the amount of mRNA in the absence of the substance indicates a gene that is modulated by Staul activity.

45. The method of claim 44, wherein the gene is up-regulated by down-regulating Staul.

46. The method of claim 44, wherein the gene is down-regulated by down-regulating Staul.

47. The method of claim 44, wherein the abundance of the gene is increased by down-regulating Staul.

48. The method of claim 44, wherein the abundance of the gene is decreased by down-regulating Staul.

49. The method of claim 44, wherein the gene is stabilized by down-regulating of Staul.

50. The method of claim 44, wherein the gene is destabilized by down-regulating of Staul.

51. A method of identifying genes modulated by the down-regulation of Staul comprising

a) transfecting a small interfering RNA (siRNA) that down-regulates SMD into a cell comprising Staul 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 Staul

activity.

52. The method of claim 51, wherein the siRNA is Staul siRNA.

53. The method of claim 51, wherein the gene is up-regulated by down-regulating Staul.

54. The method of claim 51, wherein the gene is down-regulated by down-regulating Staul.

55. The method of claim 51, wherein the abundance of the gene is increased by down-regulating Staul.

56. The method of claim 51, wherein the abundance of the gene is decreased by down-regulating Staul.

57. The method of claim 51, wherein the gene is stabilized by down-regulating of Staul.

58. The method of claim 51, wherein the gene is destabilized by down-regulating of Staul.

59. The method of claim 51, wherein the amount of mRNA present is measured by RT-PCR.

60. The method of claim 51, wherein the amount of protein expression is measured by western blotting.

61. A method of identifying genes modulated by the down-regulation of UPf1 comprising

a) transfecting a small 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 modulated by Upf1 activity.

62. The method of claim 61, wherein the siRNA is Upf1 siRNA.

63. The method of claim 61, wherein the gene is up-regulated by down-regulating Staul.

64. The method of claim 61, wherein the gene is down-regulated by down-regulating Staul.

65. The method of claim 61, wherein the abundance of the gene is increased by down-regulating Staul.

66. The method of claim 61, wherein the abundance of the gene is decreased by down-regulating Staul.

67. The method of claim 61, wherein the gene is stabilized by down-regulating of Staul.

68. The method of claim 61, wherein the gene is destabilized by down-regulating of Staul.

69. The method of claim 61, wherein the amount of mRNA present is measured by RT-PCR.

70. The method of claim 61, wherein the amount of protein expression is measured by western blotting.

71. A method of treating a disorder in a subject comprising administering to the subject a substance, wherein the substance modulates Staul wherein the modulation of Staul modulates that level of mRNA abundance of another gene.

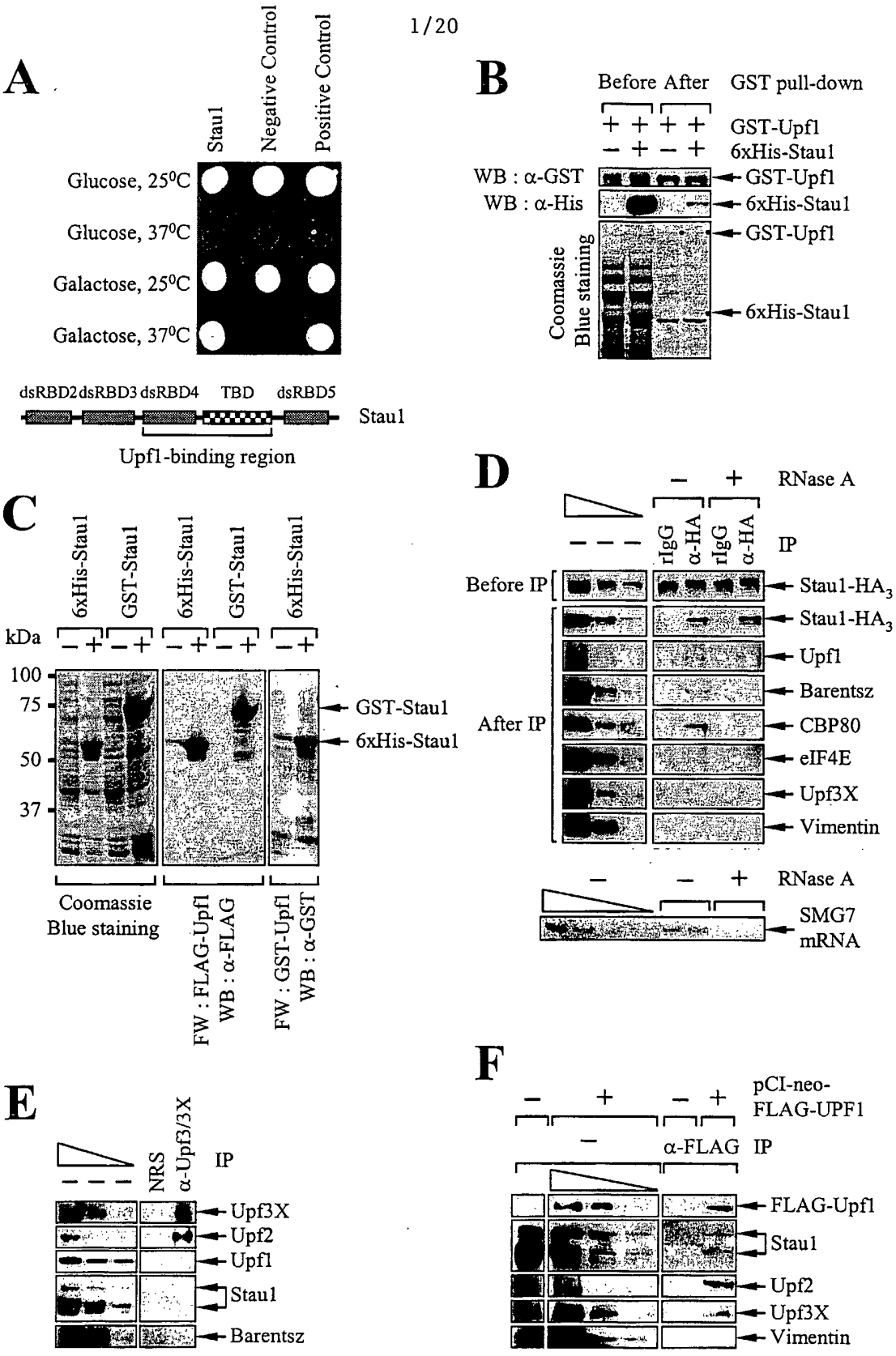
A
Stau1
Negative Control
Positive Control
Glucose, 25°C
Glucose, 37°C
Galactose, 25°C
Galactose, 37°C
dsRBD2 dsRBD3 dsRBD4 TBD dsRBD5
Stau1
Upf1-binding region
B
Before After GST pull-down
GST-Upf1
6xHis-Stau1
WB : α-GST
WB : α-His
Coomassie Blue staining
C
6xHis-Stau1
GST-Stau1
kDa
100
75
50
37
GST-Stau1
6xHis-Stau1
Coomassie Blue staining
FW : FLAG-Upf1 WB : α-FLAG
FW : GST-Upf1 WB : α-GST
D
RNase A
IP
rIgG
α-HA
Before IP
After IP
Stau1-HA3
Upf1
Barentsz
CBP80
eIF4E
Upf3X
Vimentin
SMG7 mRNA
E
NRS
α-Upf3/3X
IP
Upf3X
Upf2
Upf1
Stau1
Barentsz
F
pCI-neo-FLAG-UPF1
α-FLAG IP
FLAG-Upf1
Stau1
Upf2
Upf3X
Vimentin

Fig. 1

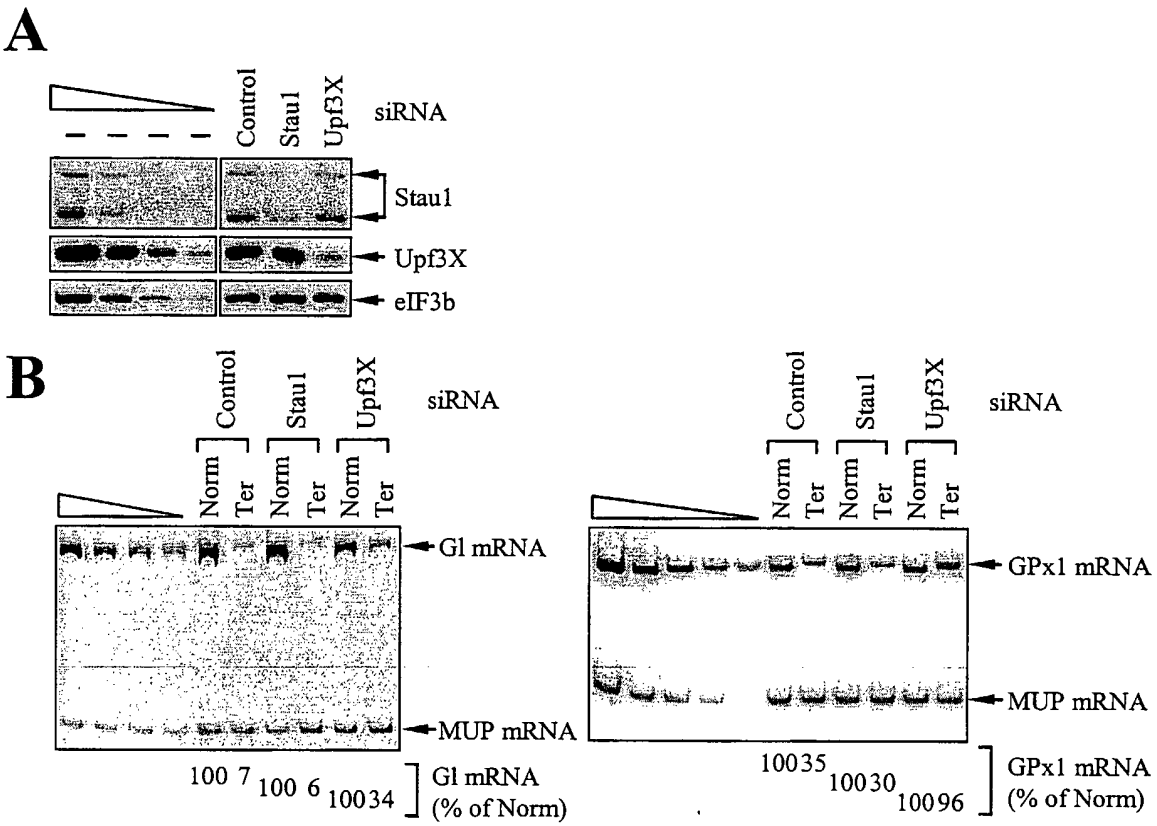
A
Control
Stau1
Upf3X
siRNA
Stau1
Upf3X
eIF3b
B
Control
Stau1
Upf3X
siRNA
Norm
Ter
Gl mRNA
MUP mRNA
100 7
100 6
10034
Gl mRNA
(% of Norm)
GPx1 mRNA
10035
10030
10096
GPx1 mRNA
(% of Norm)

Fig. 2

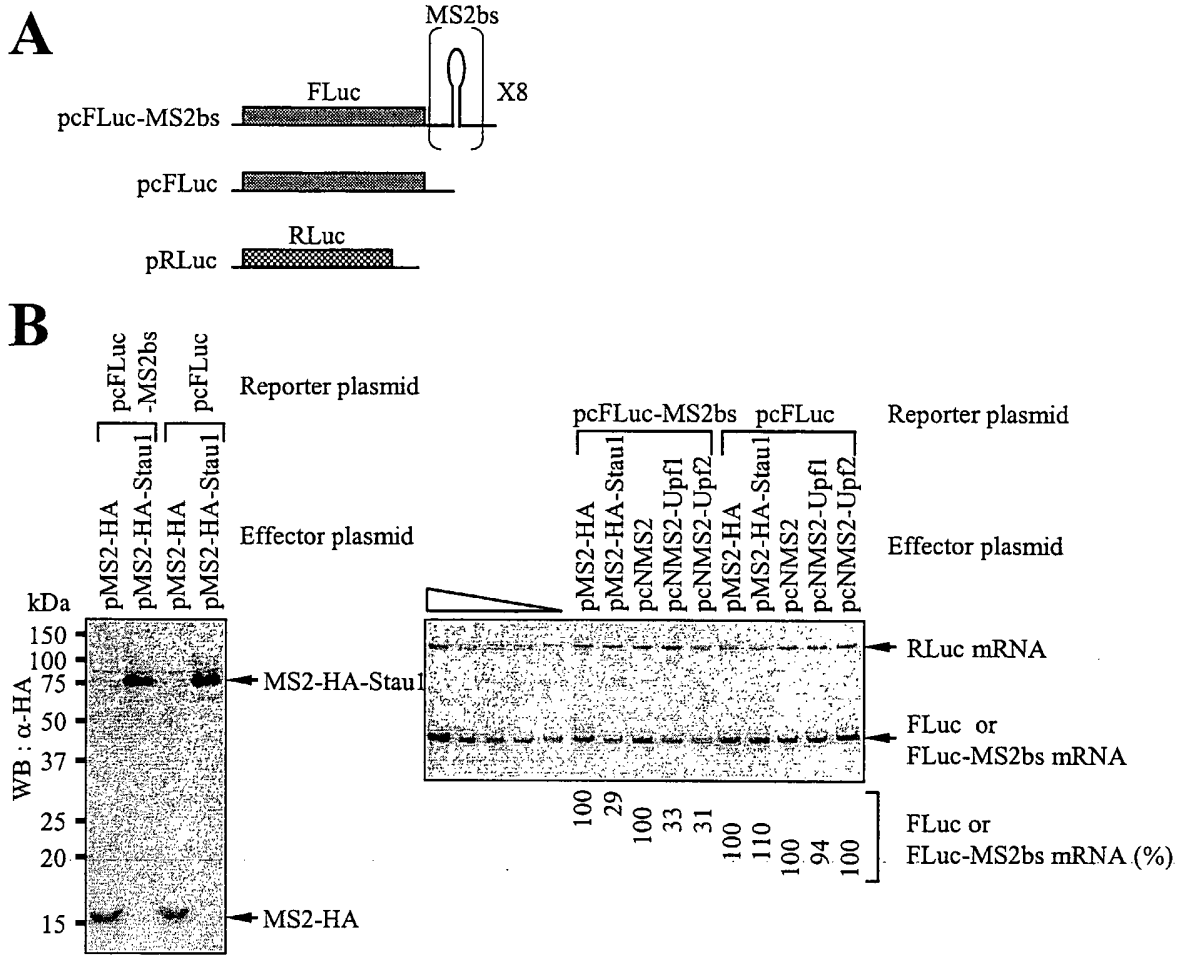
A
MS2bs
FLuc
X8
pcFLuc-MS2bs
pcFLuc
RLuc
pRLuc
B
pcFLuc-MS2bs
pcFLuc
Reporter plasmid
pMS2-HA
pMS2-HA-Stau1
pcNMS2
pcNMS2-Upf1
pcNMS2-Upf2
Effector plasmid
kDa
150
100
75
50
37
25
20
15
WB : α-HA
MS2-HA-Stau1
MS2-HA
RLuc mRNA
FLuc or
FLuc-MS2bs mRNA
100
29
100
33
31
100
110
100
94
100
FLuc or
FLuc-MS2bs mRNA (%)

Fig. 3

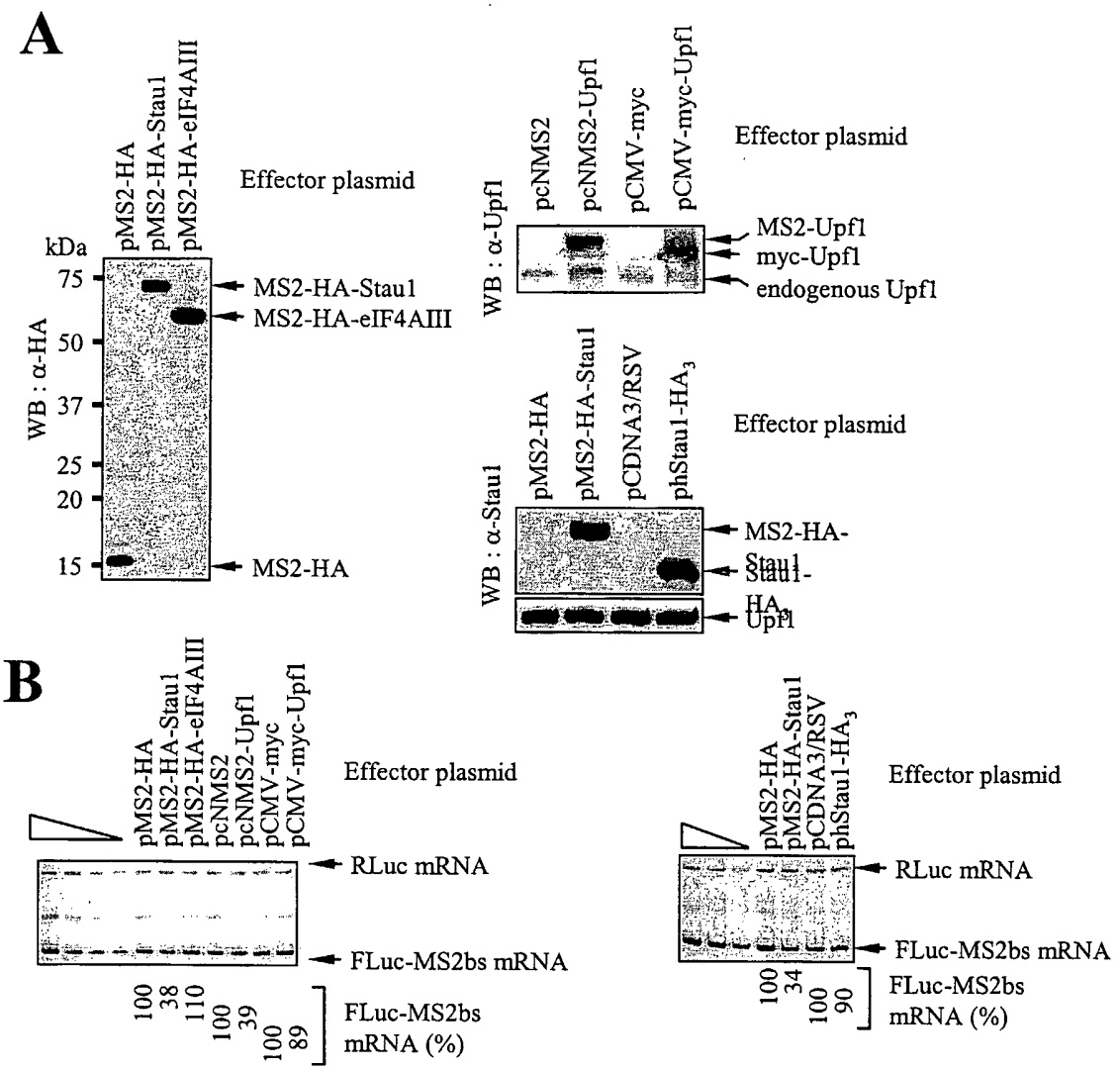
A
Effector plasmid
pMS2-HA
pMS2-HA-Stau1
pMS2-HA-eIF4AIII
kDa
75
50
37
25
20
15
WB : α-HA
MS2-HA-Stau1
MS2-HA-eIF4AIII
MS2-HA
Effector plasmid
pcNMS2
pcNMS2-Upf1
pCMV-myc
pCMV-myc-Upf1
WB : α-Upf1
MS2-Upf1
myc-Upf1
endogenous Upf1
Effector plasmid
pMS2-HA
pMS2-HA-Stau1
pCDNA3/RSV
phStau1-HA3
WB : α-Stau1
MS2-HA-Stau1
Stau1-HA3
Upf1
B
Effector plasmid
pMS2-HA
pMS2-HA-Stau1
pMS2-HA-eIF4AIII
pcNMS2
pcNMS2-Upf1
pCMV-myc
pCMV-myc-Upf1
RLuc mRNA
FLuc-MS2bs mRNA
100 38 110 100 39 100 89
FLuc-MS2bs mRNA (%)
Effector plasmid
pMS2-HA
pMS2-HA-Stau1
pCDNA3/RSV
phStau1-HA3
RLuc mRNA
FLuc-MS2bs mRNA
100 34 100 90
FLuc-MS2bs mRNA (%)

Fig. 4

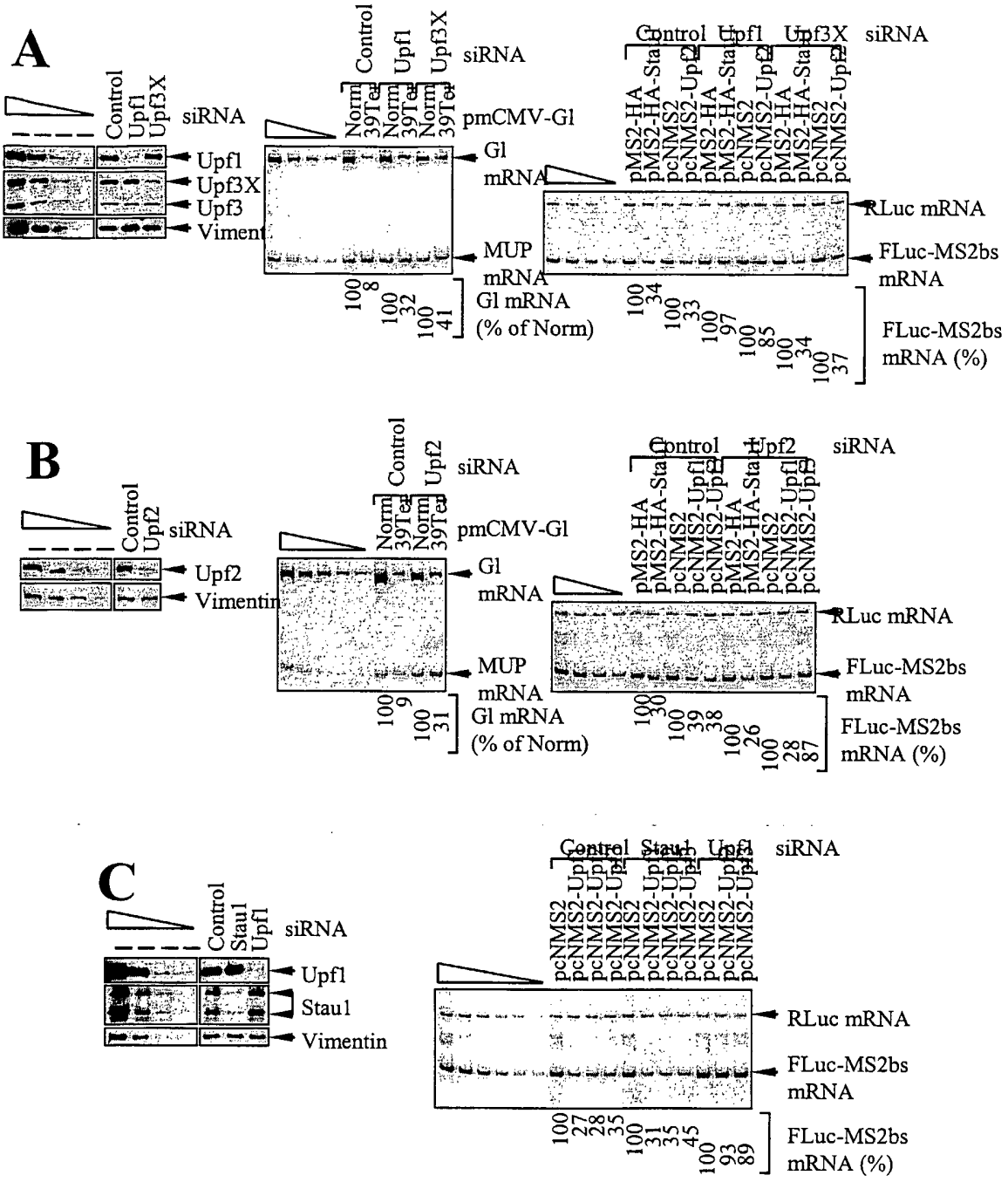
A
Control
Upf1
Upf3X
siRNA
Upf1
Upf3X
Upf3
Vimentin
Norm
39Tet
pmCMV-Gl
Gl mRNA
MUP mRNA
100 8 100 32 100 41
Gl mRNA (% of Norm)
pMS2-HA
pMS2-HA-Stau1
pcNMS2
pcNMS2-Upf1
pcNMS2-Upf2
pcNMS2-Upf3X
RLuc mRNA
FLuc-MS2bs mRNA
100 34 100 33 100 97 100 85 100 34 100 37
FLuc-MS2bs mRNA (%)
B
Control
Upf2
siRNA
Upf2
Vimentin
Norm
39Tet
pmCMV-Gl
Gl mRNA
MUP mRNA
100 9 100 31
Gl mRNA (% of Norm)
pMS2-HA
pMS2-HA-Stau1
pcNMS2
pcNMS2-Upf1
pcNMS2-Upf2
pcNMS2-Upf3X
RLuc mRNA
FLuc-MS2bs mRNA
100 30 100 39 100 26 100 28
FLuc-MS2bs mRNA (%)
C
Control
Stau1
Upf1
siRNA
Upf1
Stau1
Vimentin
pcNMS2
pcNMS2-Upf1
pcNMS2-Upf2
pcNMS2-Upf3X
pcNMS2-Stau1
RLuc mRNA
FLuc-MS2bs mRNA
100 27 28 35 100 31 35 45 100 93 89
FLuc-MS2bs mRNA (%)

Fig. 5

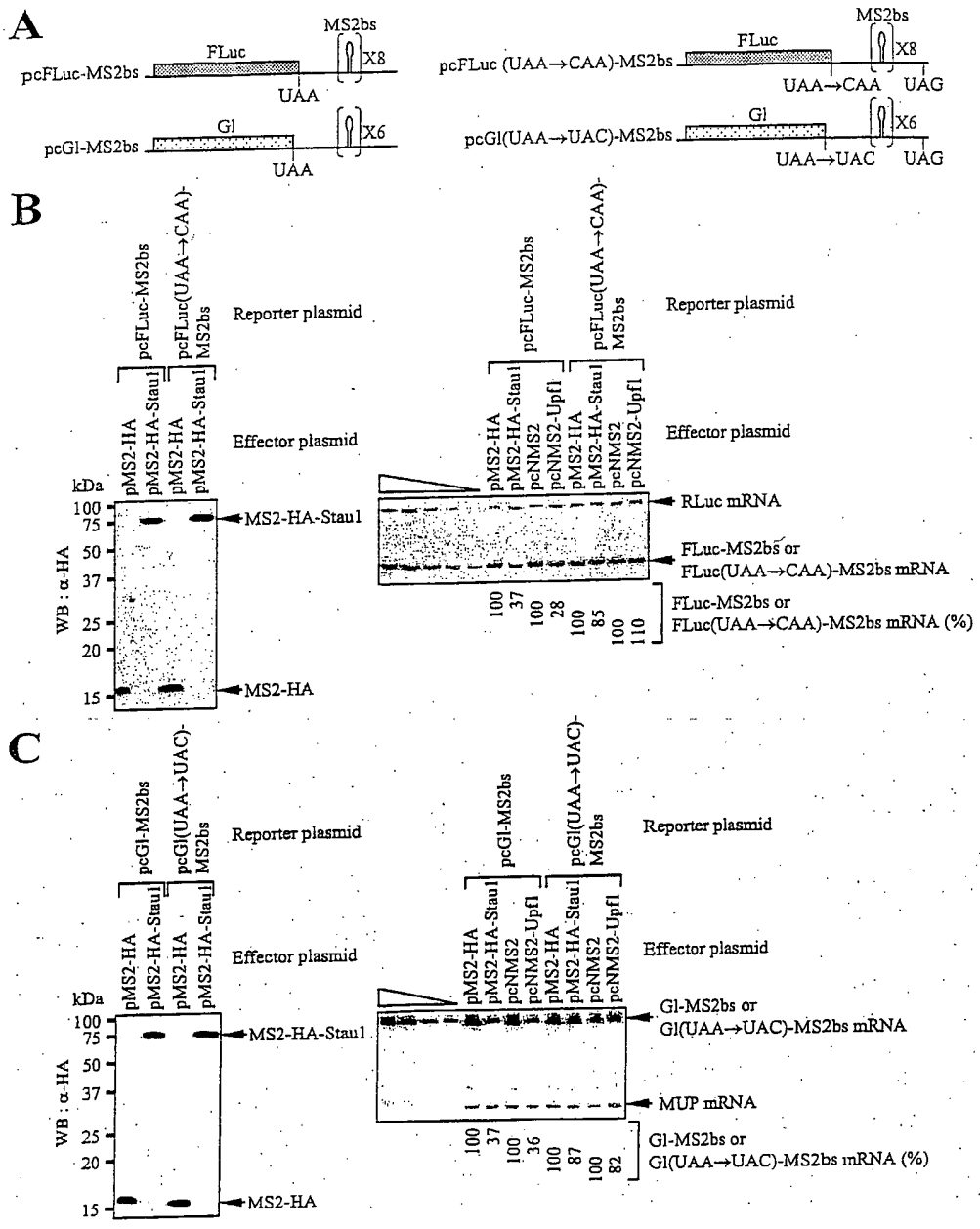
A
pcFLuc-MS2bs
FLuc
MS2bs
X8
UAA
pcGl-MS2bs
Gl
X6
UAA
pcFLuc (UAA→CAA)-MS2bs
UAA→CAA
UAG
pcGl(UAA→UAC)-MS2bs
UAA→UAC
UAG
B
Reporter plasmid
Effector plasmid
pcFLuc-MS2bs
pcFLuc(UAA→CAA)-MS2bs
pMS2-HA
pMS2-HA-Stau1
pcNMS2
pcNMS2-Upf1
kDa
WB : α-HA
MS2-HA-Stau1
MS2-HA
RLuc mRNA
FLuc-MS2bs or FLuc(UAA→CAA)-MS2bs mRNA
FLuc-MS2bs or FLuc(UAA→CAA)-MS2bs mRNA (%)
C
pcGl-MS2bs
pcGl(UAA→UAC)-MS2bs
Gl-MS2bs or Gl(UAA→UAC)-MS2bs mRNA
MUP mRNA
Gl-MS2bs or Gl(UAA→UAC)-MS2bs mRNA (%)

FIG. 6

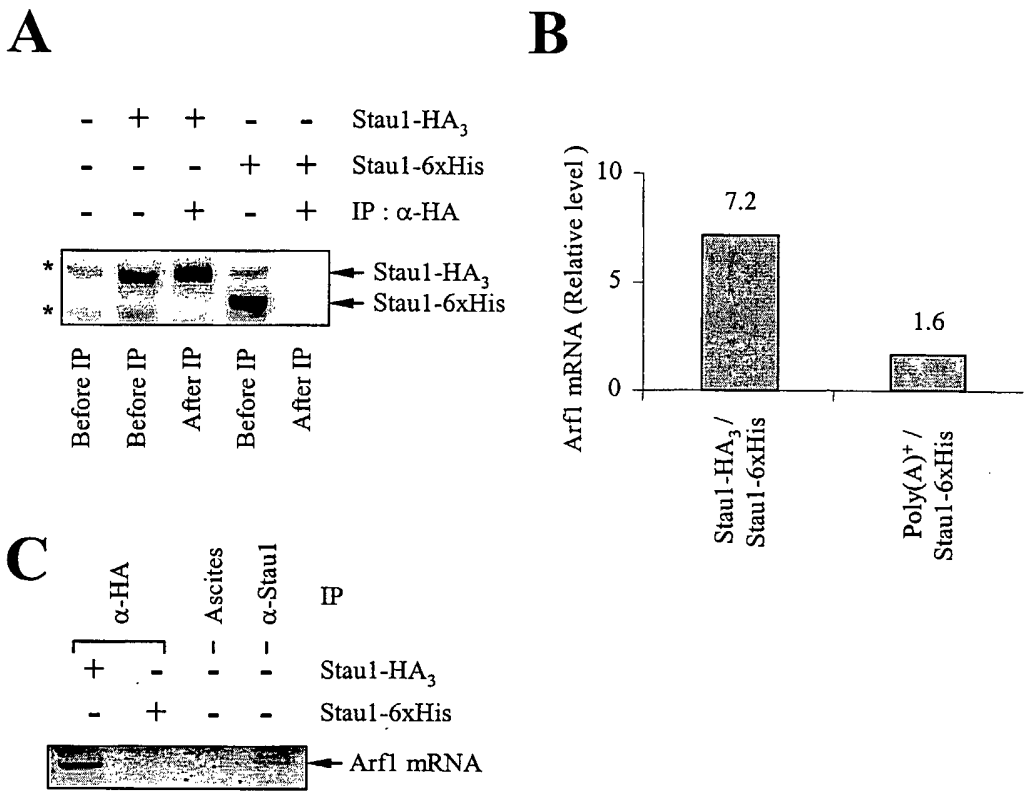
A
- + + - - Stau1-HA3
- - - + + Stau1-6xHis
- - + - + IP : α-HA
* Stau1-HA3
* Stau1-6xHis
Before IP
Before IP
After IP
Before IP
After IP
B
Arfl mRNA (Relative level)
10
5
0
7.2
1.6
Stau1-HA3 / Stau1-6xHis
Poly(A)+ / Stau1-6xHis
C
α-HA
Ascites
α-Stau1
IP
+ - - - Stau1-HA3
- + - - Stau1-6xHis
Arfl mRNA

Fig. 7

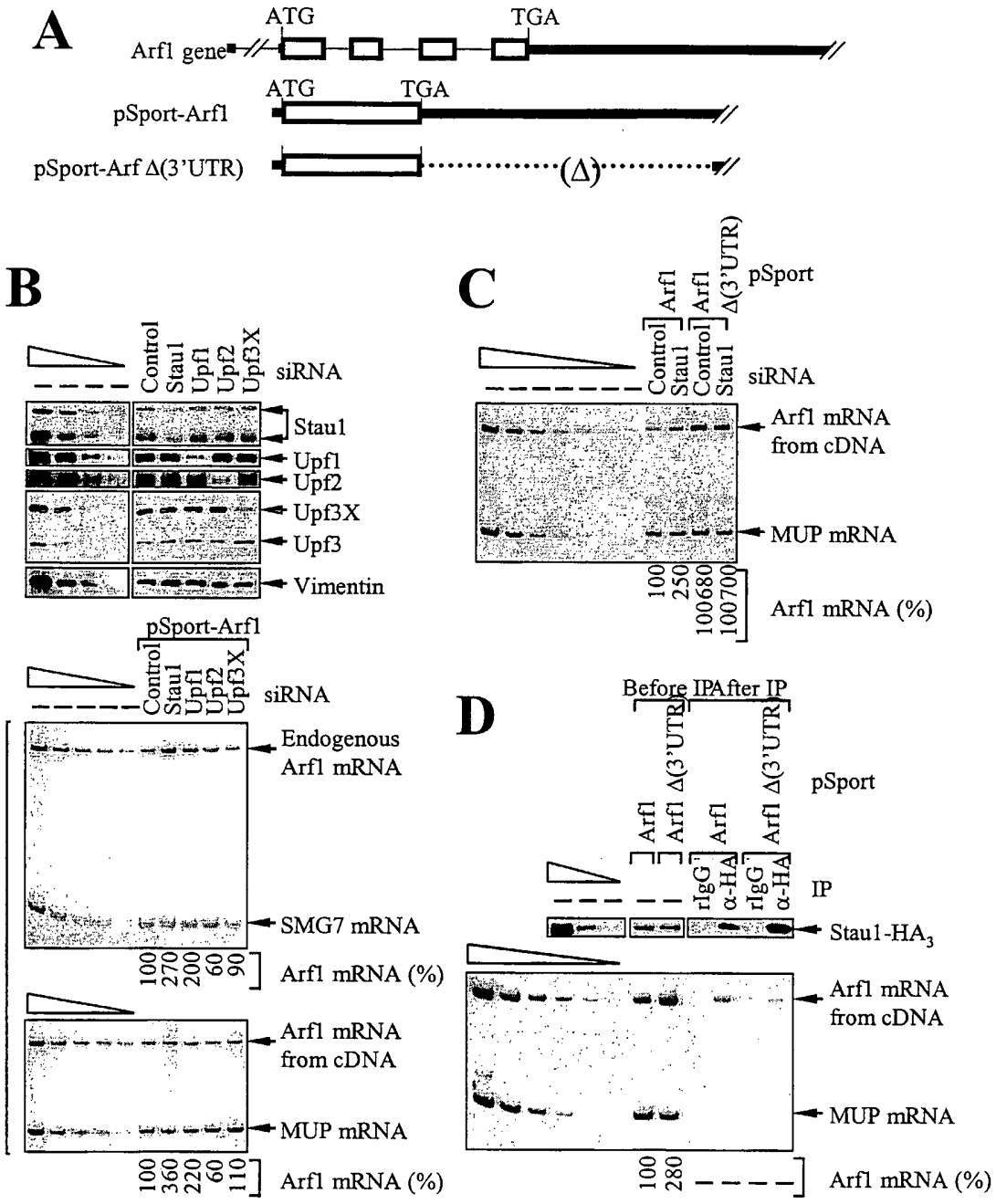
A
ATG
TGA
Arf1 gene
ATG
TGA
pSport-Arf1
pSport-Arf Δ(3'UTR)
(Δ)
B
Control
Stau1
Upf1
Upf2
Upf3X
siRNA
Stau1
Upf1
Upf2
Upf3X
Upf3
Vimentin
pSport-Arf1
Control
Stau1
Upf1
Upf2
Upf3X
siRNA
Endogenous Arf1 mRNA
SMG7 mRNA
100 270 200 60 90
Arf1 mRNA (%)
Arf1 mRNA from cDNA
MUP mRNA
100 360 220 60 110
Arf1 mRNA (%)
C
Arf1
Arf1 Δ(3'UTR)
pSport
Control
Stau1
Control
Stau1
siRNA
Arf1 mRNA from cDNA
MUP mRNA
100 250 100680 100700
Arf1 mRNA (%)
D
Before IP
After IP
Arf1
Arf1 Δ(3'UTR)
Arf1
Arf1 Δ(3'UTR)
pSport
rIgG
α-HA
rIgG
α-HA
IP
Stau1-HA3
Arf1 mRNA from cDNA
MUP mRNA
100 280
Arf1 mRNA (%)

Fig. 8

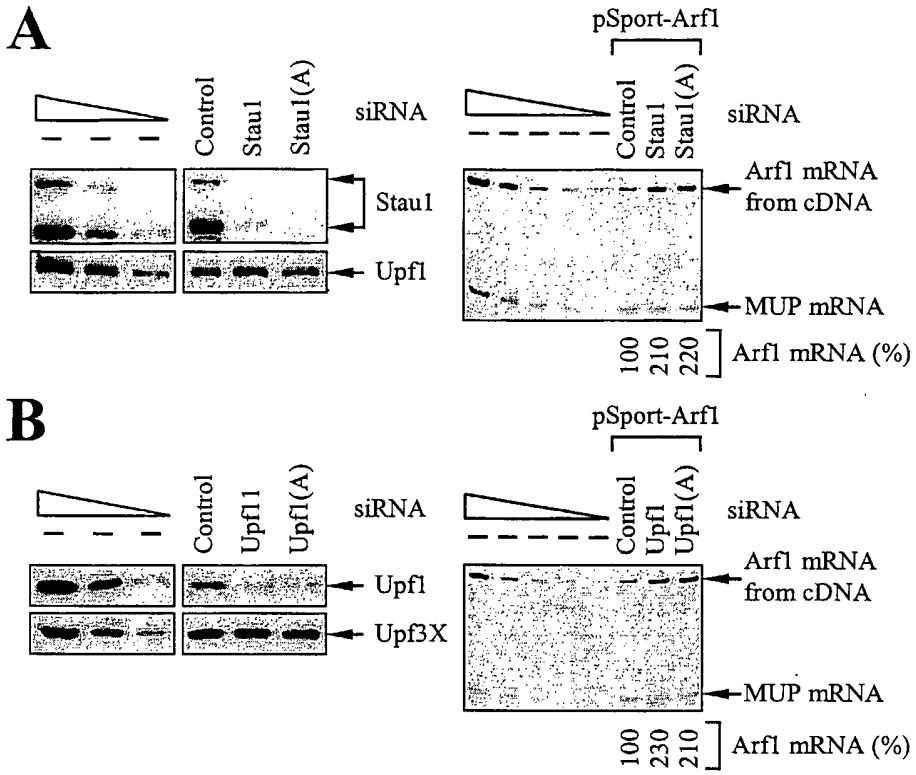
A
pSport-Arf1
Control
Stau1
Stau1(A)
siRNA
Stau1
Upf1
Arf1 mRNA from cDNA
MUP mRNA
100 210 220
Arf1 mRNA (%)
B
pSport-Arf1
Control
Upf1
Upf1(A)
siRNA
Upf1
Upf3X
Arf1 mRNA from cDNA
MUP mRNA
100 230 210
Arf1 mRNA (%)

Fig. 9

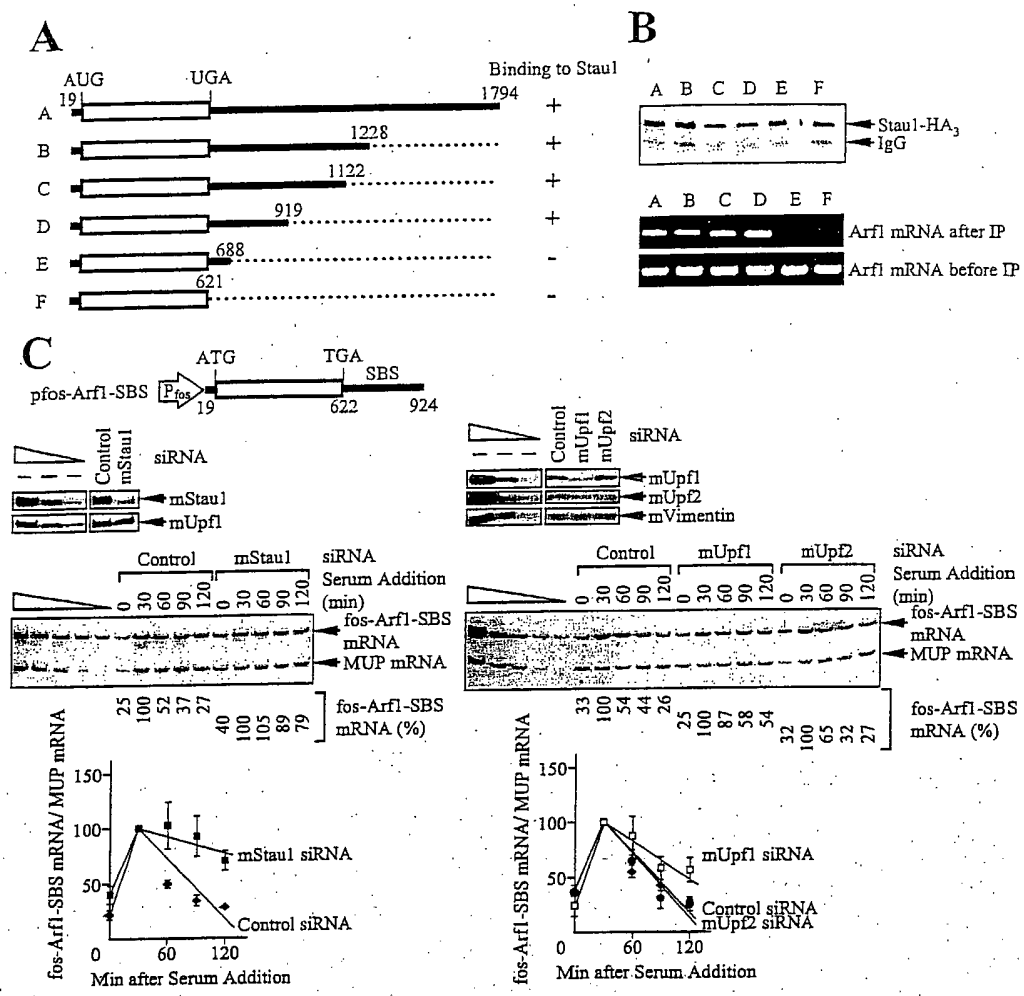
A
AUG
UGA
Binding to Stau1
19
1794
A
1228
B
1122
C
919
D
688
E
621
F
B
A B C D E F
Stau1-HA3
IgG
A B C D E F
Arf1 mRNA after IP
Arf1 mRNA before IP
C
pfos-Arf1-SBS
ATG
TGA
SBS
19
622
924
Control
mStau1
siRNA
mStau1
mUpf1
Control
mUpf1
mUpf2
siRNA
mUpf1
mUpf2
mVimentin
Control
mStau1
siRNA
Serum Addition (min)
0 30 60 90 120 0 30 60 90 120
fos-Arf1-SBS mRNA
MUP mRNA
25 100 52 37 27 40 100 105 89 79
fos-Arf1-SBS mRNA (%)
Control
mUpf1
mUpf2
siRNA
Serum Addition (min)
0 30 60 90 120 0 30 60 90 120 0 30 60 90 120
fos-Arf1-SBS mRNA
MUP mRNA
33 100 54 44 26 25 100 87 58 54 32 100 65 32 27
fos-Arf1-SBS mRNA (%)
fos-Arf1-SBS mRNA/ MUP mRNA
150
100
50
0 60 120
Min after Serum Addition
mStau1 siRNA
Control siRNA
fos-Arf1-SBS mRNA/ MUP mRNA
150
100
50
0 60 120
Min after Serum Addition
mUpf1 siRNA
Control siRNA
mUpf2 siRNA

FIG. 10

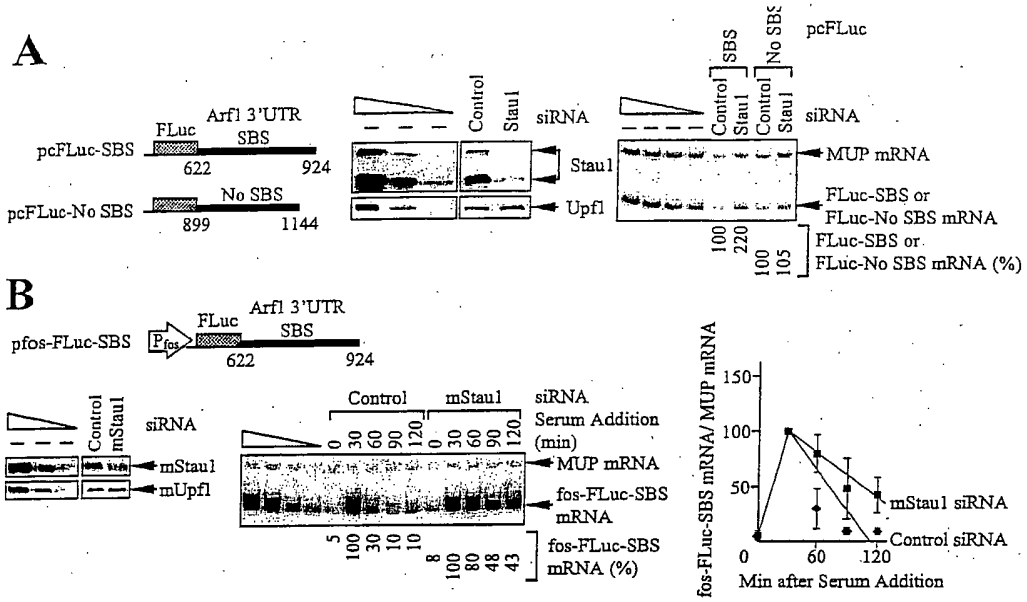
A
pcFLuc-SBS
FLuc
Arfl 3'UTR
SBS
622
924
pcFLuc-No SBS
No SBS
899
1144
Control
Stau1
siRNA
Stau1
Upfl
pcFLuc
SBS
No SBS
MUP mRNA
FLuc-SBS or
FLuc-No SBS mRNA
100
220
100
105
FLuc-SBS or
FLuc-No SBS mRNA (%)
B
pfos-FLuc-SBS
Pfos
FLuc
Arfl 3'UTR
SBS
622
924
Control
mStau1
siRNA
mStau1
mUpfl
Serum Addition
(min)
0
30
60
90
120
MUP mRNA
fos-FLuc-SBS
mRNA
5
100
30
10
10
8
100
80
48
43
fos-FLuc-SBS
mRNA (%)
fos-FLuc-SBS mRNA/ MUP mRNA
150
100
50
0
60
120
Min after Serum Addition
mStau1 siRNA
Control siRNA

FIG. 11

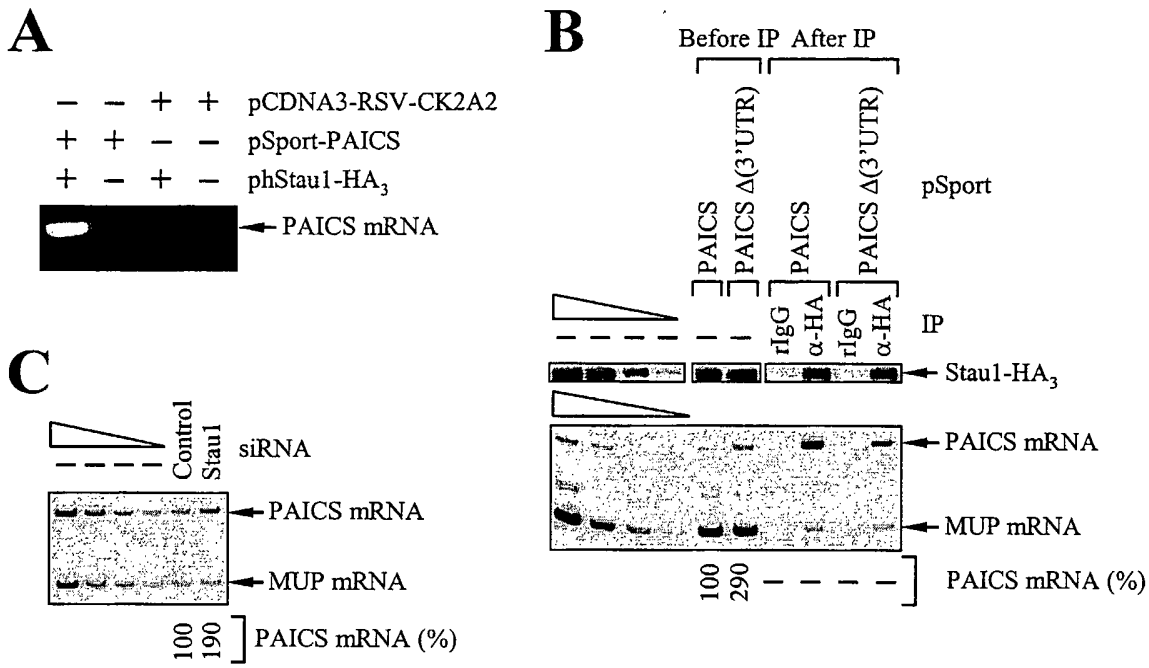
A
pCDNA3-RSV-CK2A2
pSport-PAICS
phStau1-HA3
PAICS mRNA
B
Before IP
After IP
PAICS
PAICS Δ(3'UTR)
PAICS
PAICS Δ(3'UTR)
pSport
rIgG
α-HA
rIgG
α-HA
IP
Stau1-HA3
PAICS mRNA
MUP mRNA
100
290
PAICS mRNA (%)
C
Control
Stau1
siRNA
PAICS mRNA
MUP mRNA
100
190
PAICS mRNA (%)

Fig. 12

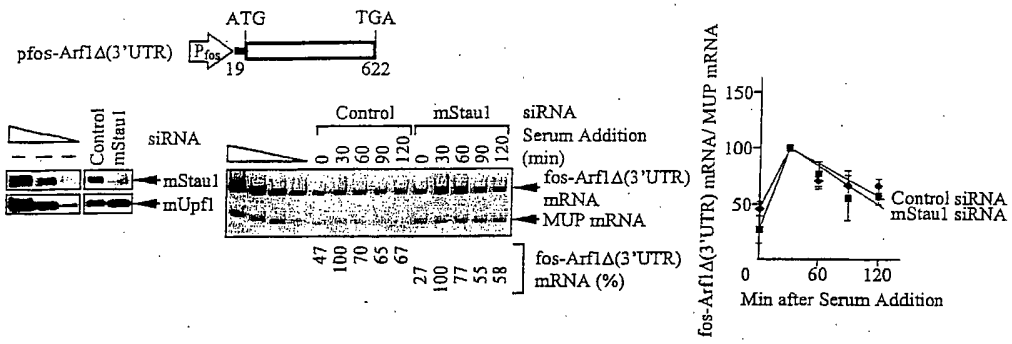
pfos-Arf1Δ(3'UTR)
ATG
TGA
19
622
Control
mStau1
siRNA
mStau1
mUpf1
Control
mStau1
siRNA
Serum Addition
(min)
0 30 60 90 120 0 30 60 90 120
fos-Arf1Δ(3'UTR)
mRNA
MUP mRNA
47 100 70 65 67 27 100 77 55 58
fos-Arf1Δ(3'UTR)
mRNA (%)
fos-Arf1Δ(3'UTR) mRNA/ MUP mRNA
150
100
50
0
60
120
Min after Serum Addition
Control siRNA
mStau1 siRNA

FIG. 13

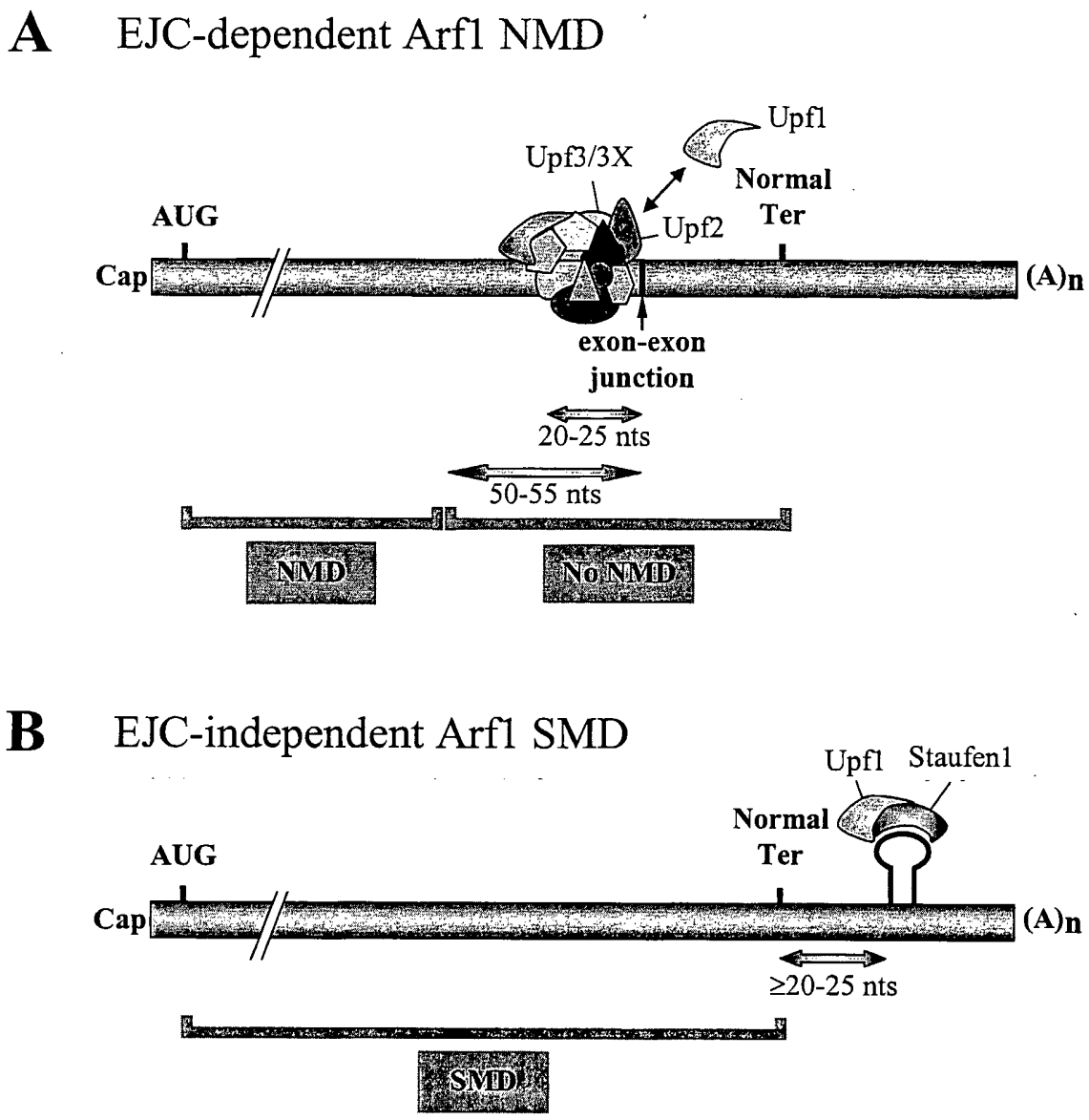
A
EJC-dependent Arf1 NMD
Upf1
Upf3/3X
Normal
Ter
AUG
Upf2
Cap
(A)n
exon-exon
junction
20-25 nts
50-55 nts
NMD
No NMD
B
EJC-independent Arf1 SMD
Upf1
Staufen1
Normal
Ter
AUG
Cap
(A)n
≥20-25 nts
SMD

Fig. 14

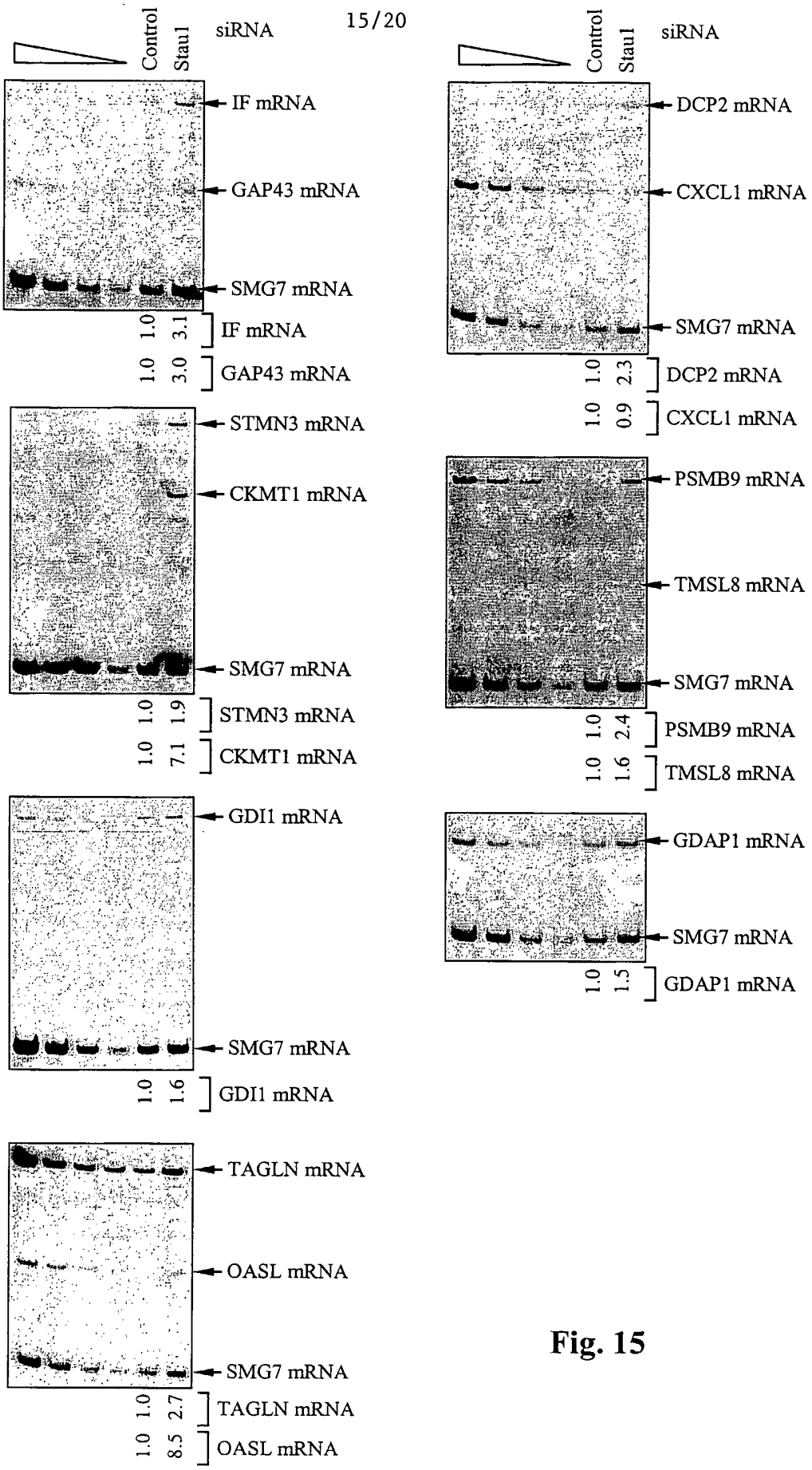
siRNA
Control
Stau1
IF mRNA
GAP43 mRNA
SMG7 mRNA
1.0 3.1 IF mRNA
1.0 3.0 GAP43 mRNA
STMN3 mRNA
CKMT1 mRNA
SMG7 mRNA
1.0 1.9 STMN3 mRNA
1.0 7.1 CKMT1 mRNA
GDI1 mRNA
SMG7 mRNA
1.0 1.6 GDI1 mRNA
TAGLN mRNA
OASL mRNA
SMG7 mRNA
1.0 1.0 2.7 TAGLN mRNA
1.0 8.5 OASL mRNA
siRNA
Control
Stau1
DCP2 mRNA
CXCL1 mRNA
SMG7 mRNA
1.0 1.0 2.3 DCP2 mRNA
1.0 0.9 CXCL1 mRNA
PSMB9 mRNA
TMSL8 mRNA
SMG7 mRNA
1.0 1.0 2.4 PSMB9 mRNA
1.0 1.6 TMSL8 mRNA
GDAP1 mRNA
SMG7 mRNA
1.0 1.5 GDAP1 mRNA

Fig. 15

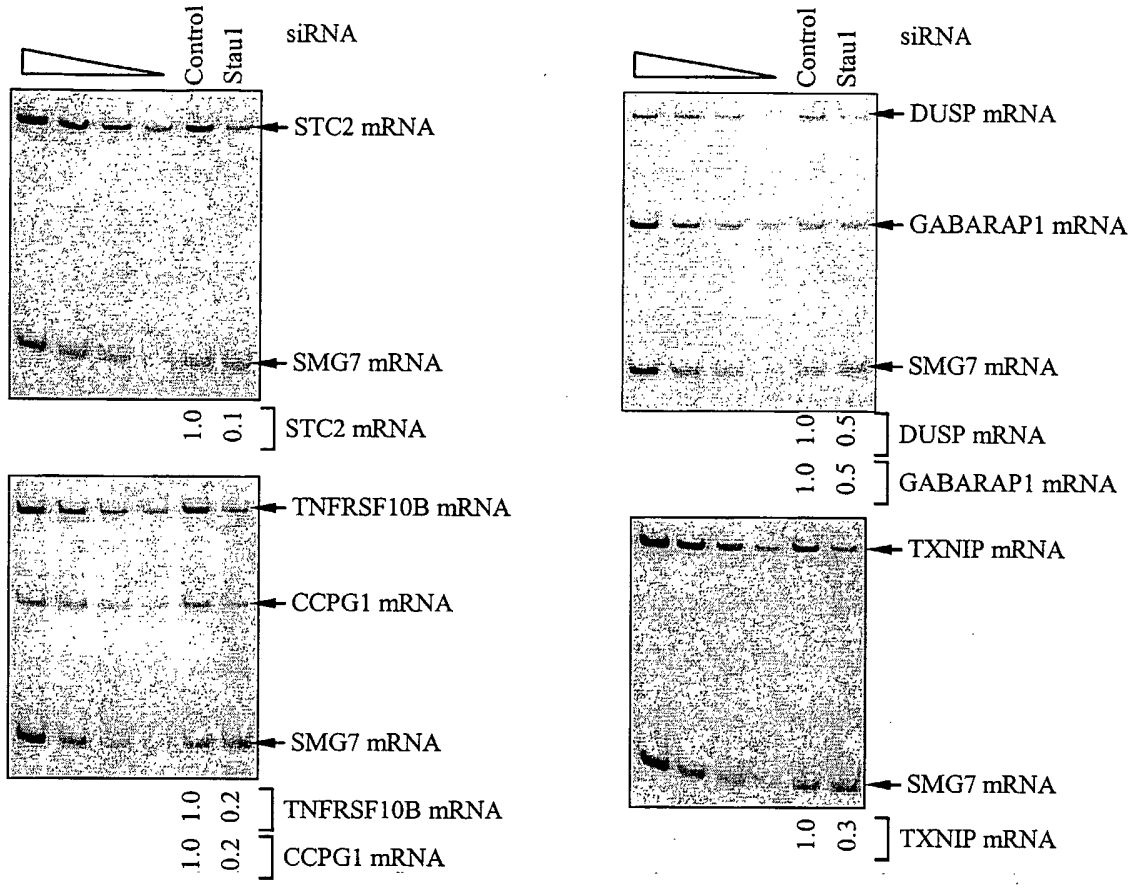
siRNA
Control
Stau1
STC2 mRNA
SMG7 mRNA
1.0 0.1
STC2 mRNA
TNFRSF10B mRNA
CCPG1 mRNA
SMG7 mRNA
1.0 1.0 0.2
TNFRSF10B mRNA
1.0 0.2
CCPG1 mRNA
siRNA
Control
Stau1
DUSP mRNA
GABARAP1 mRNA
SMG7 mRNA
1.0 1.0 0.5
DUSP mRNA
1.0 0.5
GABARAP1 mRNA
TXNIP mRNA
SMG7 mRNA
1.0 0.3
TXNIP mRNA

Fig. 16

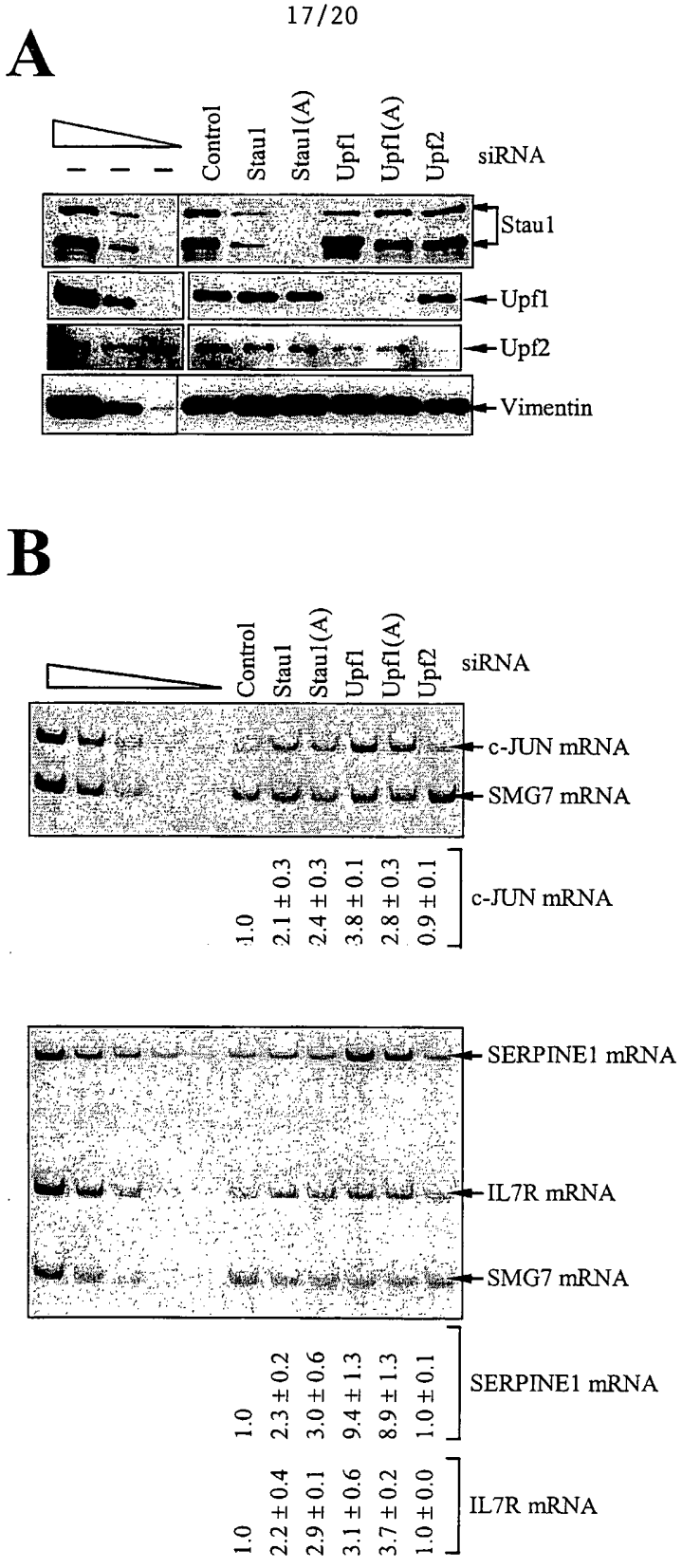
A
Control
Stau1
Stau1(A)
Upf1
Upf1(A)
Upf2
siRNA
Stau1
Upf1
Upf2
Vimentin
B
Control
Stau1
Stau1(A)
Upf1
Upf1(A)
Upf2
siRNA
c-JUN mRNA
SMG7 mRNA
1.0
2.1 ± 0.3
2.4 ± 0.3
3.8 ± 0.1
2.8 ± 0.3
0.9 ± 0.1
c-JUN mRNA
SERPINE1 mRNA
IL7R mRNA
SMG7 mRNA
1.0
2.3 ± 0.2
3.0 ± 0.6
9.4 ± 1.3
8.9 ± 1.3
1.0 ± 0.1
SERPINE1 mRNA
1.0
2.2 ± 0.4
2.9 ± 0.1
3.1 ± 0.6
3.7 ± 0.2
1.0 ± 0.0
IL7R mRNA

Fig. 17

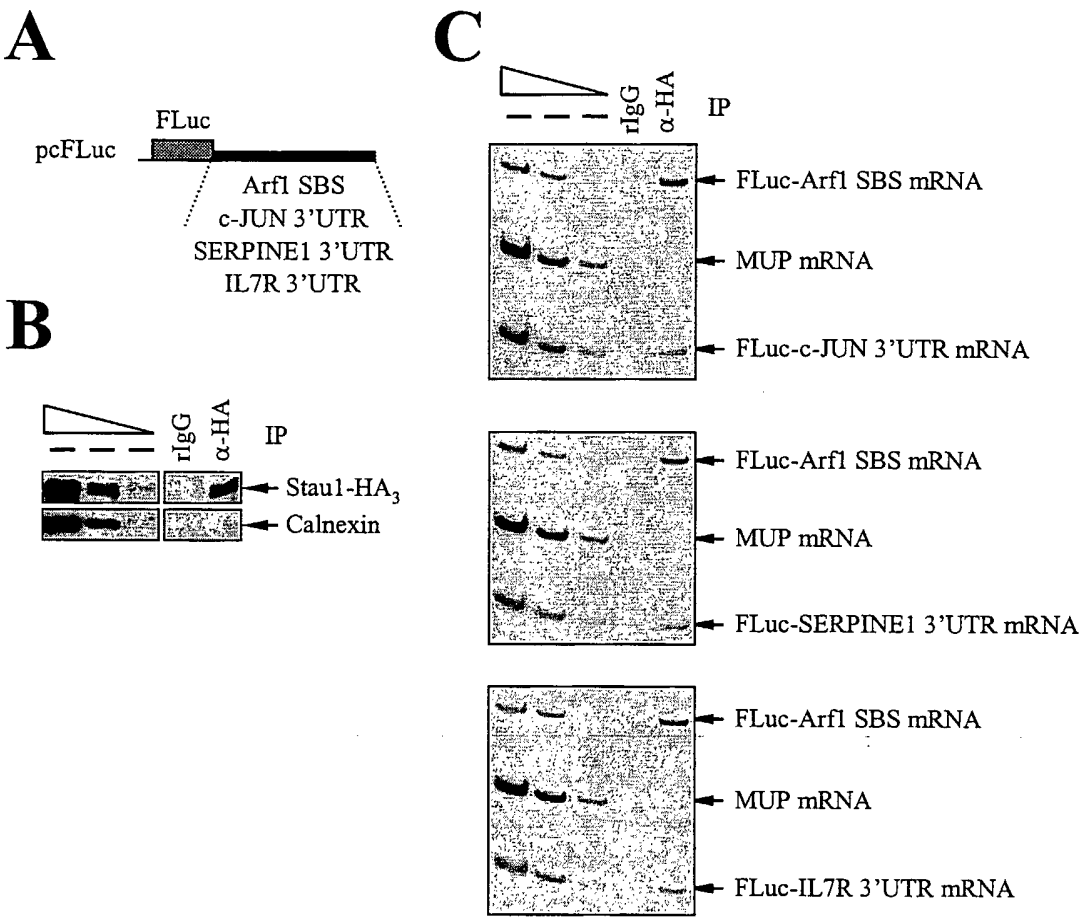
A
FLuc
pcFLuc
Arf1 SBS
c-JUN 3'UTR
SERPINE1 3'UTR
IL7R 3'UTR
B
rIgG
α-HA
IP
Stau1-HA3
Calnexin
C
rIgG
α-HA
IP
FLuc-Arf1 SBS mRNA
MUP mRNA
FLuc-c-JUN 3'UTR mRNA
FLuc-Arf1 SBS mRNA
MUP mRNA
FLuc-SERPINE1 3'UTR mRNA
FLuc-Arf1 SBS mRNA
MUP mRNA
FLuc-IL7R 3'UTR mRNA

Fig. 18

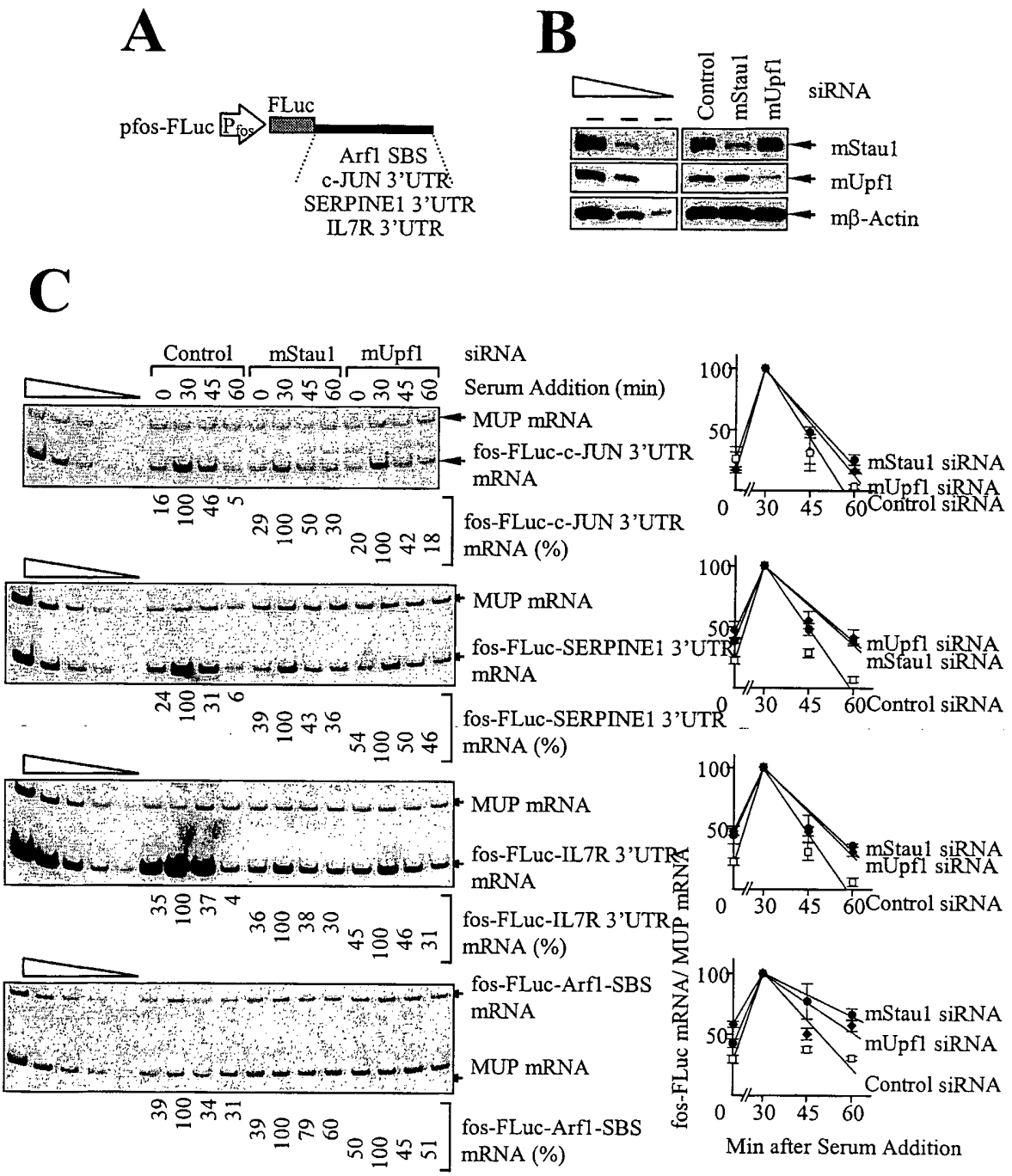
A
pfos-FLuc
FLuc
Arf1 SBS
c-JUN 3'UTR
SERPINE1 3'UTR
IL7R 3'UTR
B
Control
mStau1
mUpf1
siRNA
mStau1
mUpf1
mβ-Actin
C
Control
mStau1
mUpf1
siRNA
Serum Addition (min)
MUP mRNA
fos-FLuc-c-JUN 3'UTR mRNA
fos-FLuc-c-JUN 3'UTR mRNA (%)
fos-FLuc-SERPINE1 3'UTR mRNA
fos-FLuc-SERPINE1 3'UTR mRNA (%)
fos-FLuc-IL7R 3'UTR mRNA
fos-FLuc-IL7R 3'UTR mRNA (%)
fos-FLuc-Arf1-SBS mRNA
fos-FLuc-Arf1-SBS mRNA (%)
fos-FLuc mRNA/ MUP mRNA
mStau1 siRNA
mUpf1 siRNA
Control siRNA
Min after Serum Addition

Fig. 19

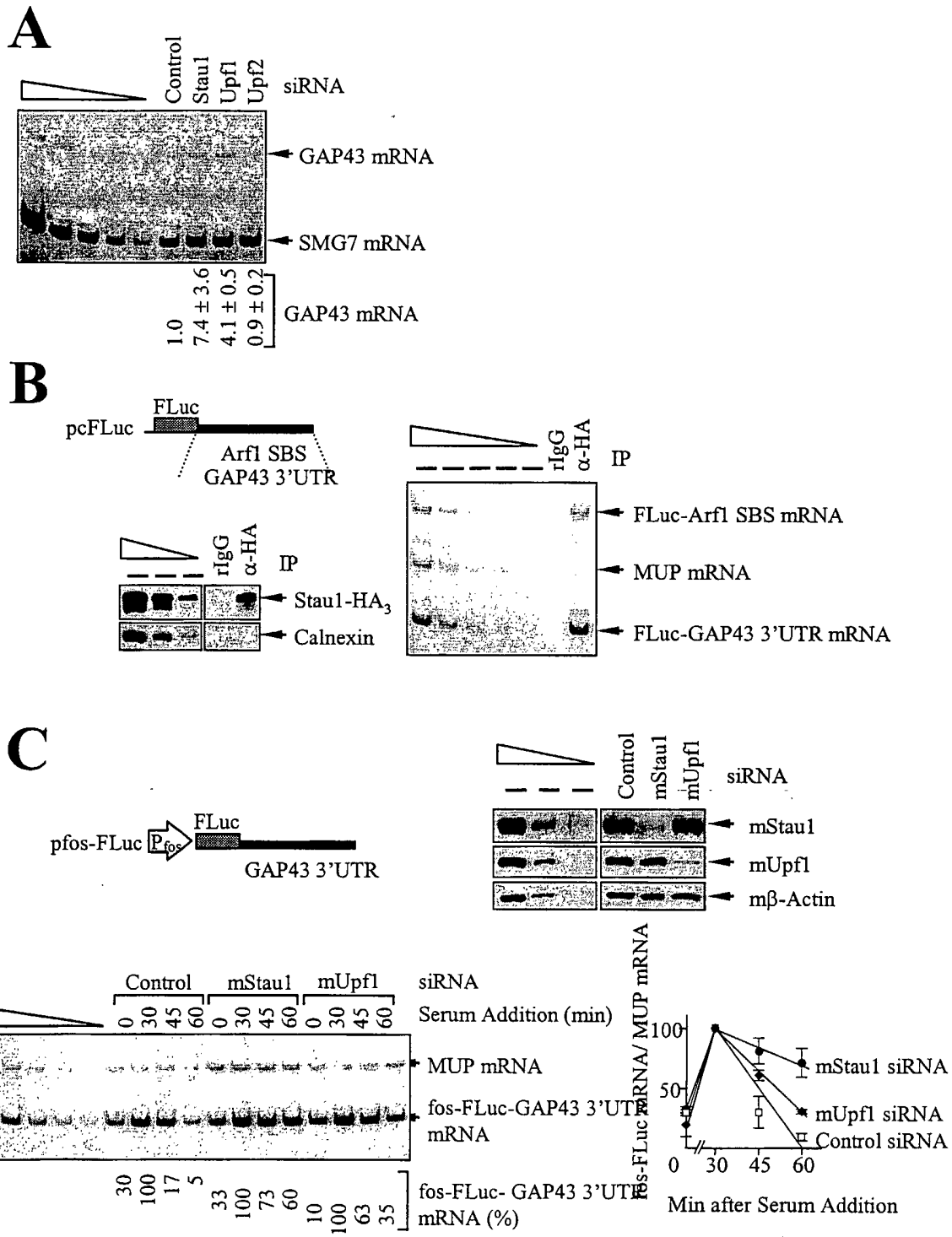
A
Control
Stau1
Upf1
Upf2
siRNA
GAP43 mRNA
SMG7 mRNA
1.0
7.4 ± 3.6
4.1 ± 0.5
0.9 ± 0.2
GAP43 mRNA
B
pcFLuc
FLuc
Arf1 SBS
GAP43 3'UTR
rIgG
α-HA
IP
Stau1-HA3
Calnexin
FLuc-Arf1 SBS mRNA
MUP mRNA
FLuc-GAP43 3'UTR mRNA
C
pfos-FLuc
Pfos
FLuc
GAP43 3'UTR
Control
mStau1
mUpf1
siRNA
mStau1
mUpf1
mβ-Actin
Control
mStau1
mUpf1
siRNA
Serum Addition (min)
0 30 45 60 0 30 45 60 0 30 45 60
MUP mRNA
fos-FLuc-GAP43 3'UTR mRNA
30 100 17 5 33 100 73 60 10 100 63 35
fos-FLuc- GAP43 3'UTR mRNA (%)
fos-FLuc mRNA/ MUP mRNA
100
50
0 30 45 60
mStau1 siRNA
mUpf1 siRNA
Control siRNA
Min after Serum Addition

Fig. 20

SEQUENCE LISTING

<110> University of Rochester

Maquat, Lynne E.
Kim, Yoon Ki

<120> STAUFEN 1 (stau 1) MEDIATED mRNA DECAY

<130> 21108.0050P1

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<151> 2005-01-25

<150> 60/614,071
<151> 2004-09-29

<160> 180

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<220>
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synthetic construct

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synthetic construct

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Gly Ser Gln Ile Leu Asn Lys Asn Gln Ser Leu Leu Ser Gln Pro Leu
            20                  25                  30
Met Ser Ile Pro Ser Thr Thr Ser Ser Leu Pro Ser Glu Asn Ala Gly
        35                  40                  45
Arg Pro Ile Gln Asn Ser Ala Leu Pro Ser Ala Ser Ile Thr Ser Thr
    50                  55                  60
Ser Ala Ala Ala Glu Ser Ile Thr Pro Thr Val Glu Leu Asn Ala Leu
65                  70                  75                  80
Cys Met Lys Leu Gly Lys Lys Pro Met Tyr Lys Pro Val Asp Pro Tyr
                85                  90                  95
Ser Arg Met Gln Ser Thr Tyr Asn Tyr Asn Met Arg Gly Gly Ala Tyr
            100                 105                 110
Pro Pro Arg Tyr Phe Tyr Pro Phe Pro Val Pro Pro Leu Leu Tyr Gln
        115                 120                 125
Val Glu Leu Ser Val Gly Gly Gln Gln Phe Asn Gly Lys Gly Lys Thr
    130                 135                 140
Arg Gln Ala Ala Lys His Asp Ala Ala Ala Lys Ala Leu Arg Ile Leu
145                 150                 155                 160
Gln Asn Glu Pro Leu Pro Glu Arg Leu Glu Val Asn Gly Arg Glu Ser
                165                 170                 175
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            180                 185                 190
Ala Leu Lys Arg Asn Leu Pro Val Asn Phe Glu Val Ala Arg Glu Ser
        195                 200                 205
Gly Pro Pro His Met Lys Asn Phe Val Thr Lys Val Ser Val Gly Glu
    210                 215                 220
Phe Val Gly Glu Gly Glu Gly Lys Ser Lys Lys Ile Ser Lys Lys Asn
225                 230                 235                 240
Ala Ala Ile Ala Val Leu Glu Glu Leu Lys Lys Leu Pro Pro Leu Pro
                245                 250                 255
Ala Val Glu Arg Val Lys Pro Arg Ile Lys Lys Lys Thr Lys Pro Ile
            260                 265                 270
Val Lys Pro Gln Thr Ser Pro Glu Tyr Gly Gln Gly Ile Asn Pro Ile
        275                 280                 285
Ser Arg Leu Ala Gln Ile Gln Gln Ala Lys Lys Glu Lys Glu Pro Glu
    290                 295                 300
Tyr Thr Leu Leu Thr Glu Arg Gly Leu Pro Arg Arg Arg Glu Phe Val
305                 310                 315                 320
Met Gln Val Lys Val Gly Asn His Thr Ala Glu Gly Thr Gly Thr Asn
                325                 330                 335
Lys Lys Val Ala Lys Arg Asn Ala Ala Glu Asn Met Leu Glu Ile Leu
            340                 345                 350
Gly Phe Lys Val Pro Gln Ala Gln Pro Thr Lys Pro Ala Leu Lys Ser
        355                 360                 365
Glu Glu Lys Thr Pro Ile Lys Lys Pro Gly Asp Gly Arg Lys Val Thr
    370                 375                 380
Phe Phe Glu Pro Gly Ser Gly Asp Glu Asn Gly Thr Ser Asn Lys Glu
385                 390                 395                 400
Asp Glu Phe Arg Met Pro Tyr Leu Ser His Gln Gln Leu Pro Ala Gly
                405                 410                 415
Ile Leu Pro Met Val Pro Glu Val Ala Gln Ala Val Gly Val Ser Gln
            420                 425                 430
Gly His His Thr Lys Asp Phe Thr Arg Ala Ala Pro Asn Pro Ala Lys
        435                 440                 445
Ala Thr Val Thr Ala Met Ile Ala Arg Glu Leu Leu Tyr Gly Gly Thr
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Ser Pro Thr Ala Glu Thr Ile Leu Lys Asn Asn Ile Ser Ser Gly His
465                 470                 475                 480
Val Pro His Gly Pro Leu Thr Arg Pro Ser Glu Gln Leu Asp Tyr Leu
                485                 490                 495
Ser Arg Val Gln Gly Phe Gln Val Glu Tyr Lys Asp Phe Pro Lys Asn
            500                 505                 510
Asn Lys Asn Glu Phe Val Ser Leu Ile Asn Cys Ser Ser Gln Pro Pro
        515                 520                 525
Leu Ile Ser His Gly Ile Gly Lys Asp Val Glu Ser Cys His Asp Met
    530                 535                 540
Ala Ala Leu Asn Ile Leu Lys Leu Leu Ser Glu Leu Asp Gln Gln Ser
545                 550                 555                 560
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Cys
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synthetic construct

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aacatattcg ggcccctggt caagctggag gccgactacg acaagaagct gaaggagtcc 1200
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aagctgttgg gccacgaggt ggaggacgta atcatcaagt gccagctgcc caagcgcttc 1620
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<210> 4
<211> 1118
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 4

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Asp Thr Glu Glu Ala Glu Leu Leu Gly Ala Asp Thr Gln Gly Ser Glu
            20                  25                  30
Phe Glu Phe Thr Asp Phe Thr Leu Pro Ser Gln Thr Gln Thr Pro Pro
        35                  40                  45
Gly Gly Pro Gly Gly Pro Gly Gly Gly Gly Ala Gly Gly Pro Gly Gly
    50                  55                  60
Ala Gly Ala Gly Ala Ala Ala Gly Gln Leu Asp Ala Gln Val Gly Pro
65                  70                  75                  80
Glu Gly Ile Leu Gln Asn Gly Ala Val Asp Asp Ser Val Ala Lys Thr
                85                  90                  95
Ser Gln Leu Leu Ala Glu Leu Asn Phe Glu Glu Asp Glu Glu Asp Thr
            100                 105                 110
Tyr Tyr Thr Lys Asp Leu Pro Ile His Ala Cys Ser Tyr Cys Gly Ile
        115                 120                 125
His Asp Pro Ala Cys Val Val Tyr Cys Asn Thr Ser Lys Lys Trp Phe
    130                 135                 140
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```
Cys Asn Gly Arg Gly Asn Thr Ser Gly Ser His Ile Val Asn His Leu
145                 150                 155                 160
Val Arg Ala Lys Cys Lys Glu Val Thr Leu His Lys Asp Gly Pro Leu
                165                 170                 175
Gly Glu Thr Val Leu Glu Cys Tyr Asn Cys Gly Cys Arg Asn Val Phe
            180                 185                 190
Leu Leu Gly Phe Ile Pro Ala Lys Ala Asp Ser Val Val Val Leu Leu
        195                 200                 205
Cys Arg Gln Pro Cys Ala Ser Gln Ser Ser Leu Lys Asp Ile Asn Trp
    210                 215                 220
Asp Ser Ser Gln Trp Gln Pro Leu Ile Gln Asp Arg Cys Phe Leu Ser
225                 230                 235                 240
Trp Leu Val Lys Ile Pro Ser Glu Gln Glu Gln Leu Arg Ala Arg Gln
                245                 250                 255
Ile Thr Ala Gln Gln Ile Asn Lys Leu Glu Glu Leu Trp Lys Glu Asn
            260                 265                 270
Pro Ser Ala Thr Leu Glu Asp Leu Glu Lys Pro Gly Val Asp Glu Glu
        275                 280                 285
Pro Gln His Val Leu Leu Arg Tyr Glu Asp Ala Tyr Gln Tyr Gln Asn
    290                 295                 300
Ile Phe Gly Pro Leu Val Lys Leu Glu Ala Asp Tyr Asp Lys Lys Leu
305                 310                 315                 320
Lys Glu Ser Gln Thr Gln Asp Asn Ile Thr Val Arg Trp Asp Leu Gly
                325                 330                 335
Leu Asn Lys Lys Arg Ile Ala Tyr Phe Thr Leu Pro Lys Thr Asp Ser
            340                 345                 350
Asp Met Arg Leu Met Gln Gly Asp Glu Ile Cys Leu Arg Tyr Lys Gly
        355                 360                 365
Asp Leu Ala Pro Leu Trp Lys Gly Ile Gly His Val Ile Lys Val Pro
    370                 375                 380
Asp Asn Tyr Gly Asp Glu Ile Ala Ile Glu Leu Arg Ser Ser Val Gly
385                 390                 395                 400
Ala Pro Val Glu Val Thr His Asn Phe Gln Val Asp Phe Val Trp Lys
                405                 410                 415
Ser Thr Ser Phe Asp Arg Met Gln Ser Ala Leu Lys Thr Phe Ala Val
            420                 425                 430
Asp Glu Thr Ser Val Ser Gly Tyr Ile Tyr His Lys Leu Leu Gly His
        435                 440                 445
Glu Val Glu Asp Val Ile Ile Lys Cys Gln Leu Pro Lys Arg Phe Thr
    450                 455                 460
Ala Gln Gly Leu Pro Asp Leu Asn His Ser Gln Val Tyr Ala Val Lys
465                 470                 475                 480
Thr Val Leu Gln Arg Pro Leu Ser Leu Ile Gln Gly Pro Pro Gly Thr
                485                 490                 495
Gly Lys Thr Val Thr Ser Ala Thr Ile Val Tyr His Leu Ala Arg Gln
            500                 505                 510
Gly Asn Gly Pro Val Leu Val Cys Ala Pro Ser Asn Ile Ala Val Asp
        515                 520                 525
Gln Leu Thr Glu Lys Ile His Gln Thr Gly Leu Lys Val Val Arg Leu
    530                 535                 540
Cys Ala Lys Ser Arg Glu Ala Ile Asp Ser Pro Val Ser Phe Leu Ala
545                 550                 555                 560
Leu His Asn Gln Ile Arg Asn Met Asp Ser Met Pro Glu Leu Gln Lys
                565                 570                 575
Leu Gln Gln Leu Lys Asp Glu Thr Gly Glu Leu Ser Ser Ala Asp Glu
            580                 585                 590
Lys Arg Tyr Arg Ala Leu Lys Arg Thr Ala Glu Arg Glu Leu Leu Met
        595                 600                 605
Asn Ala Asp Val Ile Cys Cys Thr Cys Val Gly Ala Gly Asp Pro Arg
    610                 615                 620
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Leu Ala Lys Met Gln Phe Arg Ser Ile Leu Ile Asp Glu Ser Thr Gln
625                 630                 635                 640
Ala Thr Glu Pro Glu Cys Met Val Pro Val Val Leu Gly Ala Lys Gln
                645                 650                 655
Leu Ile Leu Val Gly Asp His Cys Gln Leu Gly Pro Val Val Met Cys
            660                 665                 670
Lys Lys Ala Ala Lys Ala Gly Leu Ser Gln Ser Leu Phe Glu Arg Leu
        675                 680                 685
Val Val Leu Gly Ile Arg Pro Ile Arg Leu Gln Val Gln Tyr Arg Met
    690                 695                 700
His Pro Ala Leu Ser Ala Phe Pro Ser Asn Ile Phe Tyr Glu Gly Ser
705                 710                 715                 720
Leu Gln Asn Gly Val Thr Ala Ala Asp Arg Val Lys Lys Gly Phe Asp
                725                 730                 735
Phe Gln Trp Pro Gln Pro Asp Lys Pro Met Phe Phe Tyr Val Thr Gln
            740                 745                 750
Gly Gln Glu Glu Ile Ala Ser Ser Gly Thr Ser Tyr Leu Asn Arg Thr
        755                 760                 765
Glu Ala Ala Asn Val Glu Lys Ile Thr Thr Lys Leu Leu Lys Ala Gly
    770                 775                 780
Ala Lys Pro Asp Gln Ile Gly Ile Ile Thr Pro Tyr Glu Gly Gln Arg
785                 790                 795                 800
Ser Tyr Leu Val Gln Tyr Met Gln Phe Ser Gly Ser Leu His Thr Lys
                805                 810                 815
Leu Tyr Gln Glu Val Glu Ile Ala Ser Val Asp Ala Phe Gln Gly Arg
            820                 825                 830
Glu Lys Asp Phe Ile Ile Leu Ser Cys Val Arg Ala Asn Glu His Gln
        835                 840                 845
Gly Ile Gly Phe Leu Asn Asp Pro Arg Arg Leu Asn Val Ala Leu Thr
    850                 855                 860
Arg Ala Arg Tyr Gly Val Ile Ile Val Gly Asn Pro Lys Ala Leu Ser
865                 870                 875                 880
Lys Gln Pro Leu Trp Asn His Leu Leu Asn Tyr Tyr Lys Glu Gln Lys
                885                 890                 895
Val Leu Val Glu Gly Pro Leu Asn Asn Leu Arg Glu Ser Leu Met Gln
            900                 905                 910
Phe Ser Lys Pro Arg Lys Leu Val Asn Thr Ile Asn Pro Gly Ala Arg
        915                 920                 925
Phe Met Thr Thr Ala Met Tyr Asp Ala Arg Glu Ala Ile Ile Pro Gly
    930                 935                 940
Ser Val Tyr Asp Arg Ser Ser Gln Gly Arg Pro Ser Ser Met Tyr Phe
945                 950                 955                 960
Gln Thr His Asp Gln Ile Gly Met Ile Ser Ala Gly Pro Ser His Val
                965                 970                 975
Ala Ala Met Asn Ile Pro Ile Pro Phe Asn Leu Val Met Pro Pro Met
            980                 985                 990
Pro Pro Pro Gly Tyr Phe Gly Gln Ala Asn Gly Pro Ala Ala Gly Arg
        995                 1000                1005
Gly Thr Pro Lys Gly Lys Thr Gly Arg Gly Gly Arg Gln Lys Asn Arg
    1010                1015                1020
Phe Gly Leu Pro Gly Pro Ser Gln Thr Asn Leu Pro Asn Ser Gln Ala
1025                1030                1035                1040
Ser Gln Asp Val Ala Ser Gln Pro Phe Ser Gln Gly Ala Leu Thr Gln
                1045                1050                1055
Gly Tyr Ile Ser Met Ser Gln Pro Ser Gln Met Ser Gln Pro Gly Leu
            1060                1065                1070
Ser Gln Pro Glu Leu Ser Gln Asp Ser Tyr Leu Gly Asp Glu Phe Lys
        1075                1080                1085
Ser Gln Ile Asp Val Ala Leu Ser Gln Asp Ser Thr Tyr Gln Gly Glu
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<210> 5
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 5
gaagatctag aatgaaactt ggaaaaaaac caatgtataa g 41

<210> 6
<211> 46
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 6
ataagaatgc ggccgctcag cacctcccac acacagacat tggtcc 46

<210> 7
<211> 46
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 7
cgctactagc tagccgccat ggcttctaac tttactcagt tcgttc 46

<210> 8
<211> 75
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 8
ataagaatgc ggccgcctag gatccagcgt agtctggaac gtcgtatggg tagatgccgg 60
agtttgctgc gattg 75

<210> 9
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 9
aggtctagat cggagcagca gcctctgagg tgt 33

<210> 10
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 10
ggctcgagct aatagctata attacagtgc ttgtttgtcg aaatg 45

<210> 11
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 11
gctatttagg tgacactata gaaggtac 28

<210> 12
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 12
ccgctcgagt tcacttctgg ttccggagct gattg 35

<210> 13
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 13
catttcgaca aacaagcact gtaattatag ctattag 37

<210> 14
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 14
ccaaggacaa gcgagttgcg 20

<210> 15
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 15
cgactggcat ccaggccgta ac 22

<210> 16
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 16
gtgcccatgg gcctacatcc 20

<210> 17
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 17
acgagccgca cgtttgcca 19

<210> 18
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 18
gttcacttct ggttccggag ctg 23

<210> 19
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 19
acaaccatgg ggaacatctt cgccaacctc ttc 33

<210> 20
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 20
ccggaattct gggcctacat cccctctcag cactgaac 38

<210> 21
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
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synthetic construct

<400> 21
cgcggatcca tggcgaccac ggccacgatg gcgacc 36

<210> 22
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 22
ataagaatgc ggccgctcag ataagatcag caacgttcat cgg 43

<210> 23
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 23
ttgaccgctt gaagtcttta attaaatac 29

<210> 24
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 24
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<210> 25
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 25
gctctagagt gaccgaattc gtgaacgcga ccccccctccc tctcactc 48

<210> 26
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 26
gctctagagg gcccaggtgc ccatgggcct acatcccc 38

<210> 27
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 27
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<210> 28
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 28
gctctagagg gcccgagggg aacagctggg ctggcgactg g 41

<210> 29
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<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 29
ccaagcttac gcgtacccag ctttc 25

<210> 30
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 30
cccctaaaaa attcaatggc attctttc 28

<210> 31
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
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synthetic construct

<220>
<221> misc_feature
<222> (1)...(19)
<223> RNA

<220>
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<222> (20)...(21)
<223> n = t (DNA)

<400> 31
gaugcaguuc cgcuccauun n 21

<210> 32
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<220>
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<222> (1)...(19)
<223> RNA

<220>
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<222> (20)...(21)
<223> n = t (DNA)
<400> 32
ggcuuuuguc ccagccaucn n 21

<210> 33
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<212> RNA

<213> Artificial Sequence

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synthetic construct

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<221> misc_feature
<222> (1)...(19)
<223> RNA

<220>
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<222> (20)...(21)
<223> n = t (DNA)

<400> 33
ggagaagcga guaacccugn n 21

<210> 34
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<220>
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<222> (1)...(19)
<223> RNA

<220>
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<222> (20)...(21)
<223> n = t (DNA)

<400> 34
ccuauaacua caacaugagn n 21

<210> 35
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<220>
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<222> (1)...(19)
<223> RNA

<220>
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<222> (20)...(21)
<223> n = t (DNA)

<400> 35
guuugagauu gcacuuaaan n 21

<210> 36
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<212> RNA
<213> Artificial Sequence

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<222> (1)...(19)
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<220>
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<222> (20)...(21)
<223> n = t (DNA)

<400> 36
aacguuugcc guggaugagn n 21

<210> 37
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<212> RNA
<213> Artificial Sequence

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synthetic construct

<220>
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<222> (1)...(19)
<223> RNA

<220>
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<222> (20)...(21)
<223> n = t (DNA)

<400> 37
caacuguacu accuuuccan n 21

<210> 38
<211> 21
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<213> Artificial Sequence

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synthetic construct

<220>
<221> misc_feature
<222> (1)...(19)
<223> RNA

<220>
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<222> (20)...(21)
<223> n = t (DNA)

<400> 38
aacgguaacu gccaugauan n 21

<210> 39
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 39
atgacttcga aagtttatg 19

<210> 40
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 40
ttcagatttg atcaacgca 19

<210> 41
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 41
aaccaacgcc tggctcgg 18

<210> 42
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 42
agtccttcat agagcccgtc g 21

<210> 43
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 43
gctatttagg tgacactata gaaggtac 28

<210> 44
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 44
ttcttgtact ccacggtttc 20

<210> 45
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 45
gctatttagg tgacactata gaaggtac 28

<210> 46
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 46
ctctgtcatt gctgtccacc acg 23

<210> 47
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 47
caacacccca acatcttcg 19

<210> 48
<211> 19
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: note =
synthetic construct

<400> 48
ctttccgccc ttcttggcc 19

<210> 49
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 49
aatacgactc actataggga 20

<210> 50
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 50
gatacttgtg ggccagggca 20

<210> 51
<211> 19

<212> DNA
<213> Artificial Sequence

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<223> Description of Artificial Sequence: note =
synthetic construct

<400> 51
tctagaggat agaatggcg 19

<210> 52
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<212> DNA
<213> Artificial Sequence

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<223> Description of Artificial Sequence: note =
synthetic construct

<400> 52
agcaggctgg taccggtccg 20

<210> 53
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 53
accaatgttc agtacctcag 20

<210> 54
<211> 1814
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 54
gcaaaaccaa cgcctggctc ggagcagcag cctctgaggt gtccctggcc agtgtccttc 60
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aaagaaatgc gcatcctcat ggtgggcctg gatgctgcag ggaagaccac gatcctctac 180
aagcttaagc tgggtgagat cgtgaccacc attcccacca taggcttcaa cgtggaaacc 240
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cccctgtggc gccactactt ccagaacaca caaggcctga tcttcgtggt ggacagcaat 360
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gcggccgaga tcacagacaa gctggggctg cactcactac gccacaggaa ctggtacatt 540
caggccacct gcgccaccag cggcgacggg ctctatgaag gactggactg gctgtccaat 600
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cctgcaaggg tccgatttgc catcgaaaaa gacaacctct actttttttct tttgtatttt 1620
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atctcttaac tatt 1814

<210> 55
<211> 181
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 55
Met Gly Asn Ile Phe Ala Asn Leu Phe Lys Gly Leu Phe Gly Lys Lys
1 5 10 15

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Glu Met Arg Ile Leu Met Val Gly Leu Asp Ala Ala Gly Lys Thr Thr
            20                  25                  30
Ile Leu Tyr Lys Leu Lys Leu Gly Glu Ile Val Thr Thr Ile Pro Thr
        35                  40                  45
Ile Gly Phe Asn Val Glu Thr Val Glu Tyr Lys Asn Ile Ser Phe Thr
    50                  55                  60
Val Trp Asp Val Gly Gly Gln Asp Lys Ile Arg Pro Leu Trp Arg His
65                  70                  75                  80
Tyr Phe Gln Asn Thr Gln Gly Leu Ile Phe Val Val Asp Ser Asn Asp
                85                  90                  95
Arg Glu Arg Val Asn Glu Ala Arg Glu Glu Leu Met Arg Met Leu Ala
            100                 105                 110
Glu Asp Glu Leu Arg Asp Ala Val Leu Leu Val Phe Ala Asn Lys Gln
        115                 120                 125
Asp Leu Pro Asn Ala Met Asn Ala Ala Glu Ile Thr Asp Lys Leu Gly
    130                 135                 140
Leu His Ser Leu Arg His Arg Asn Trp Tyr Ile Gln Ala Thr Cys Ala
145                 150                 155                 160
Thr Ser Gly Asp Gly Leu Tyr Glu Gly Leu Asp Trp Leu Ser Asn Gln
                165                 170                 175
Leu Arg Asn Gln Lys
            180
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<210> 56
<211> 303
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 56

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gcggctcgtg gtgtgagtgc cagaagctgc ctccgtggtt tggtcaccgt gtgcatcgca     120
ccgtgctgta aatgtggcag acgcagcctg cggccaggct ttttatttaa tgtaaatagt     180
ttttgtttcc aatgaggcag tttctggtac tcctatgcaa tattactcag ctttttttat     240
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ctg                                                                   303
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<210> 57
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 57
acaaccatgg ggaacatctt cgccaacctc ttc 33

<210> 58
<211> 38
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: note =
synthetic construct

<400> 58
ccggaattct gggcctacat cccctctcag cactgaac 38

<210> 59
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 59
ccggaattct cacttctggt tccggagctg attggac 37

<210> 60
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<220>
<221> misc_feature
<222> (1)...(19)
<223> RNA

<220>
<221> misc_feature
<222> (20)...(21)
<223> n = t (DNA)

<400> 60
ucaagguucc ugauaauuan n 21

<210> 61
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<220>
<221> misc_feature
<222> (1)...(19)
<223> RNA

<220>
<221> misc_feature
<222> (20)...(21)
<223> n = t (DNA)

<400> 61
gaaacuucuu gaugaacaan n 21

<210> 62
<211> 21
<212> RNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<220>
<221> misc_feature
<222> (1)...(19)
<223> RNA

<220>
<221> misc_feature
<222> (20)...(21)
<223> n = t (DNA)

<400> 62
agaggcacuu cgagauaaan n 21

<210> 63
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 63
cgctctagag tgagaactct ttctggcctg ccttcgttaa c 41

<210> 64
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 64
cgctctagat tacaaatggt aaactcagag tgctcc 36

<210> 65
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 65
cgctctagag tgaccctggg gaaagacgcc ttcatctg 38

<210> 66
<211> 36

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 66
cgctctagag cttctattag attacattca tttcac 36

<210> 67
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 67
cgcgctagcg tgaagtgtaa gaaacccaga ctgaac 36

<210> 68
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 68
cgcgctagct ttttttcctc tcatgctctc ttcctgc 37

<210> 69
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 69
catgccatgg aagacgccaa aaacataaag aaaggc 36

<210> 70
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 70
cggaattcag gctgatcagc gagctctagc atttagg 37

<210> 71
<211> 33
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 71
cgctctagag tgattcgtta actgtgtatg tac 33

<210> 72
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 72
cgctctagaa aattaaaaaa tatatatatg 30

<210> 73
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 73
cgctctagag tgagatgaaa gctgattact gtc 33

<210> 74
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 74
cgctctagaa acttacaaag gcatgaagc 29

<210> 75
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 75
cgctctagag tgaatttctt gtttgtttgt ttggg 35

<210> 76
<211> 30
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 76
cgctctagat gctccaaatc tcttatttac 30

<210> 77
<211> 33
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<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 77
cgctctagag tgatgaccct ggggaaagac gcc 33

<210> 78
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 78
cgctctagag ttgggccaca tgatggggg 29

<210> 79
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<212> DNA
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<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 79
cgctctagag tgatctcctg gcctggccat ctc 33

<210> 80
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<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 80
cgctctagag gtcaatttcc atcaagggg 29

<210> 81
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<223> Description of Artificial Sequence: note =
synthetic construct

<400> 81
cgctctagag tgaatacaat ttcatcctcc ttc 33

<210> 82
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<223> Description of Artificial Sequence: note =
synthetic construct

<400> 82
cgctctagat gtctctctca cccacccc 29

<210> 83
<211> 33
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<223> Description of Artificial Sequence: note =
synthetic construct

<400> 83
cgctctagag tgaggcagct cggattcaac tac 33

<210> 84
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<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 84
cgctctagac atttcacatc tgtgtgcaa 29

<210> 85
<211> 34
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 85
cgctctagag tgaactctaa gaaatggctt tcca 34

<210> 86
<211> 33
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: note =
synthetic construct

<400> 86
cgctctagat gtttgacttg ggatctttcc tgc 33

<210> 87
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 87
cgctctagag tgaaagtcaa acagtgtggc ttaaaca 37

<210> 88
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 88
cgctctagag aattcactcg atattttgga ctcctc 36

<210> 89
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 89
cgcgctagcg tgaagtgtaa gaaacccaga ctgaac 36

<210> 90
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 90
cgcgctagca actcaggaag agtggtcaaa g 31

<210> 91
<211> 34
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: note =
synthetic construct

<400> 91
cgcgctagcg tgacagtggc actcaacatg agtc 34

<210> 92
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 92
cgcgctagct ttttttcctc tcatgctctc ttcctgc 37

<210> 93
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 93
aatacgactc actataggga 20

<210> 94
<211> 20
<212> DNA
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<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 94
aggcaggcca gaaagagttc 20

<210> 95
<211> 20
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<223> Description of Artificial Sequence: note =
synthetic construct

<400> 95
tgaaggcgtc tttccccagg 20

<210> 96
<211> 20
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: note =
synthetic construct

<400> 96
tcagtctggg tttcttacac 20

<210> 97
<211> 20
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<223> Description of Artificial Sequence: note =
synthetic construct

<400> 97
gcctggccgc aggctgcgtc 20

<210> 98
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<223> Description of Artificial Sequence: note =
synthetic construct

<400> 98
agactgagcc gatcccgcgc 20

<210> 99
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<223> Description of Artificial Sequence: note =
synthetic construct

<400> 99
cttgaaagct cagaactcgg 20

<210> 100
<211> 20
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 100
tcagcccccg acggtctctc 20

<210> 101
<211> 20
<212> DNA
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<223> Description of Artificial Sequence: note =
synthetic construct

<400> 101
accgccaatc gcaaggcacc 20

<210> 102
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 102
gctgatctca tccttgttcc 20

<210> 103
<211> 20
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<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 103
aagtggctat gctcaaaatg 20

<210> 104
<211> 20
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 104
ttcaggcact ttacctccac 20

<210> 105
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 105
ccttgacctt gggtttcaac 20

<210> 106
<211> 20
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: note =
synthetic construct

<400> 106
atttgcaatg gaagcctttg 20

<210> 107
<211> 20
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 107
tgtgctgtat gagaagaacc 20

<210> 108
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 108
gcttcatcct tcttattagc 20

<210> 109
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 109
gactggccag atgctcgtgg 20

<210> 110
<211> 20
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<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 110
atctttggga agcggctatc 20

<210> 111
<211> 20
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: note =
synthetic construct

<400> 111
ccatggccag caccatttcc 20

<210> 112
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<212> DNA
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<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 112
acctcggccg cgtgcagctc 20

<210> 113
<211> 20
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 113
tccttcctgc gagccctgag 20

<210> 114
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 114
gcactgctgc catgtctttg 20

<210> 115
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 115
tgcaatcatt gaggattgtg 20

<210> 116
<211> 20
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<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: note =
synthetic construct

<400> 116
cactgtcaag tggatgtctc 20

<210> 117
<211> 20
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<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 117
gacagagacg tgaagcactg 20

<210> 118
<211> 20
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<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 118
ccataaatgt tgctttatcc 20

<210> 119
<211> 20
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 119
cctggtagcc gctggccggc 20

<210> 120
<211> 20
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<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 120
cttctggtca gttggatttg 20

<210> 121
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<223> Description of Artificial Sequence: note =
synthetic construct

<400> 121
gatttatgtt gttgtagttg 20

<210> 122
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 122
ccaagcagcc aattttattg 20

<210> 123
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 123
acagcctttc acgagtcttc 20

<210> 124
<211> 20
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: note =
synthetic construct

<400> 124
ctgctgttgg gaggcgatcc 20

<210> 125
<211> 20
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 125
gcgggagaag tccacaccgg 20

<210> 126
<211> 20
<212> DNA
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<220>

<223> Description of Artificial Sequence: note =
synthetic construct

<400> 126
aggctgtcga gtcagcattc 20

<210> 127
<211> 20
<212> DNA
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<223> Description of Artificial Sequence: note =
synthetic construct

<400> 127
agttaactgt ggactccatg 20

<210> 128
<211> 20
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<223> Description of Artificial Sequence: note =
synthetic construct

<400> 128
actttctcca actcatcaag 20

<210> 129
<211> 20
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<223> Description of Artificial Sequence: note =
synthetic construct

<400> 129
cctgtccctg cagaatacag 20

<210> 130
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<223> Description of Artificial Sequence: note =
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<400> 130
gttcacgagg tccacgtagg 20

<210> 131
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<223> Description of Artificial Sequence: note =
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<400> 131
gcaagtcttc ttcctcaaag 20

<210> 132
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<223> Description of Artificial Sequence: note =
synthetic construct

<400> 132
ttcctccagc attcttgatg 20

<210> 133
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<223> Description of Artificial Sequence: note =
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<400> 133
aaatatccgg acagggtccc 20

<210> 134
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<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 134
tagtcttcct catgattgtc 20

<210> 135
<211> 20
<212> DNA
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<220>
<223> Description of Artificial Sequence: note =
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<400> 135
tctggaagac cagccaacag 20

<210> 136
<211> 20
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: note =
synthetic construct

<400> 136
tcagcatgga tggaaatctc 20

<210> 137
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
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<400> 137
tcgccgcggt cctgctgttg 20

<210> 138
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 138
tgaccatccc tctgggacac 20

<210> 139
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 139
ggtctggact gatgaaaatc 20

<210> 140
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: note =
synthetic construct

<400> 140
cactgtgcca ttttggctgc 20

<210> 141
<211> 38
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence; note =
synthetic construct

<400> 141
cgctctagag tgaactctaa gaaatggctt tccacatc 38

<210> 142
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 142
cgctctagag tgagaattca ctcgatattt tggactcctc ag 42

<210> 143
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 143
gctatttagg tgacactata gaaggtac 28

<210> 144
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 144
ctttttacaa taaaaaaagc tgagtaatat 30

<210> 145
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 145
tgcctcattg gaaacaaaaa ctatttacat 30

<210> 146
<211> 22
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence; note =
synthetic construct

<400> 146
aggctgcgtc tgccacattt ac 22

<210> 147
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 147
accacggagg cagcttctgg 20

<210> 148
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 148
atgagagtaa agcagagggc aag 23

<210> 149
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<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 149
catttcgaca aacaagcact gtaattatag ctattag 37

<210> 150
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 150
gttcacttct ggttccggag ctg 23

<210> 151
<211> 19
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence; note =
synthetic construct

<400> 151
ccagaagctg cctccgtgg 19

<210> 152
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 152
aagaggagtg agagggaggg 20

<210> 153
<211> 30
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<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 153
tttttgtttc caatgaggca gtttctggta 30

<210> 154
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 154
aagaggagtg agagggaggg 20

<210> 155
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 155
gctatttagg tgacactata gaaggtac 28

<210> 156
<211> 29
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence; note =
synthetic construct

<400> 156
cataggagta ccacttcctg cctcattgg 29

<210> 157
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 157
ccacggaggc agaaagtggc actcacacc 29

<210> 158
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 158
ccaatgaggc aggaagtggt actcctatg 29

<210> 159
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 159
ggtgtgagtg ccactttctg cctccgtgg 29

<210> 160
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 160
gtgcccatgg gcctacatcc 20

<210> 161
<211> 28
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence; note =
synthetic construct

<400> 161
gctatttagg tgacactata gaaggtac 28

<210> 162
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 162
aattctcgag aggcagcttc tggcactc 28

<210> 163
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 163
aattctcgag aggcagtttc tggtactcct atg 33

<210> 164
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 164
gtgcccatgg gcctacatcc 20

<210> 165
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 165
cgctctagag tgattcgtta actgtgtatg tac 33

<210> 166
<211> 30
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence; note =
synthetic construct

<400> 166
cgctctagaa aattaaaaaa tatatatatg 30

<210> 167
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 167
cgctctagag tgagatgaaa gctgattact gtc 33

<210> 168
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 168
cgctctagaa acttacaaag gcatgaagc 29

<210> 169
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 169
cgctctagag tgaatttctt gtttgtttgt ttggg 35

<210> 170
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 170
cgctctagat gctccaaatc tcttatttac 30

<210> 171
<211> 38
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence; note =
synthetic construct

<400> 171
cgctctagag tgaccctggg gaaagacgcc ttcatctg 38

<210> 172
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 172
cgctctagat gtctctctca cccacccccc cctcac 36

<210> 173
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 173
gctctagagt gatgaccctg gggaaagacg cc 32

<210> 174
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 174
cgctctagag ttgggccaca tgatggggg 29

<210> 175
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 175
cgctctagag tgatctcctg gcctggccat ctc 33

<210> 176
<211> 29
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence; note =
synthetic construct

<400> 176
cgctctagag gtcaatttcc atcaagggg 29

<210> 177
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 177
cgctctagag tgaatacaat ttcatcctcc ttc 33

<210> 178
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 178
cgctctagat gtctctctca cccacccc 29

<210> 179
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 179
cgctctagag cttctattag attacattca tttcac 36

<210> 180
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence; note =
synthetic construct

<400> 180
cgctctagag tgaggcagct cggattcaac taccttag 38