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A viral Sm-class RNA base-pairs with mRNAs and recruits miRNAs to inhibit apoptosis

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Abstract

Viruses express several classes of non-coding (nc) RNAs¹. For most of them, the functions and mechanisms by which they act are unknown. *Herpesvirus saimiri* (HVS), a γ -herpesvirus that establishes latency in T cells of New World primates and has the ability to cause aggressive leukemias and lymphomas in non-natural hosts², expresses seven small nuclear (sn), U-rich ncRNAs called HSURs in latently infected cells^{3–5}. HSURs associate with Sm proteins and share biogenesis and structural features with cellular Sm-class snRNAs^{4,6}. One of these viral snRNAs, HSUR 2, base-pairs with two host microRNAs (miRNAs), miR-142-3p and miR-16⁷. However, HSUR 2 does not affect the abundance or activity of these two cellular miRNAs, suggesting alternative functions for these interactions. Here we show that HSUR 2 also base-pairs with messenger RNAs (mRNAs) in infected cells. We combined *in vivo* psoralen-mediated RNA-RNA crosslinking and high-throughput sequencing to identify mRNAs targeted by HSUR 2. HSUR 2 targets include mRNAs encoding Retinoblastoma (pRb) and factors involved in p53 signaling and apoptosis. We show that HSUR 2 represses expression of target mRNAs. Base-pairing between HSUR 2 and miR-142-3p and miR-16 is essential for HSUR 2-mediated mRNA repression, suggesting that HSUR 2 recruits these two cellular miRNAs to target mRNAs. Furthermore, we show that HSUR 2 utilizes this mechanism to inhibit apoptosis. Our results uncover a role for a viral Sm-class RNA as a miRNA adaptor in post pre-mRNA-processing regulation of gene expression.

Since their discovery three decades ago⁸, the functions of the seven Sm-class RNAs expressed by *Herpesvirus saimiri* (HSURs) have remained elusive. HSURs 1 and 2 are conserved among all strains of HVS and are the only ones expressed by the related *Herpesvirus ateles*⁹. HSURs 1 and 2 resemble 3' untranslated regions (UTRs) of mRNAs since they contain AU-rich elements (AREs)^{10–12} and binding sites for host miRNAs

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miR-27, miR-142-3p, and miR-16⁷ (Extended data Fig. 1a). Binding of HSUR 1 to miR-27 results in degradation of this miRNA and enhancement of the activated state of the infected T cell^{7,13}. However, HSURs 1 and 2 do not affect the activity of ARE binding proteins in virally transformed T cells¹⁰ nor do they affect the abundance or activity of miR-142-3p and miR-16⁷ (Extended data Fig. 1b), suggesting that these two viral Sm-class RNAs do not function as inhibitors of miR-142-3p and miR-16.

We hypothesized that HSUR 2 regulates gene expression by base-pairing with target mRNAs, and by recruiting ARE-binding proteins, miR-142-3p, and miR-16 to such target mRNAs. We first used *in vivo* psoralen-mediated crosslinking of RNA-RNA interactions¹⁴ to determine if HSUR 2 base-pairs with mRNAs in virally-transformed marmoset T cells. Crosslinking with the psoralen derivative 4'-aminomethyltrioxalen (AMT) followed by polyadenylated (polyA+) RNA selection showed that HSUR 2 base-pairs with polyA+ RNA *in vivo* (Fig. 1a) whereas cellular spliceosomal Sm-class RNAs do not detectably do so. Hence, HSUR 2 likely targets mature mRNAs, rather than pre-mRNAs.

MiRNAs associate with actively translated mRNAs, as reflected by sucrose-gradient co-sedimentation with polyribosomes^{15,16}. Fractionation of cytoplasmic extracts from HVS-transformed marmoset T cells revealed that HSUR 2 co-sediments with polyribosomes, mirroring the sedimentation of HSUR 2-binding miR-142-3p and miR-16, whereas spliceosomal U2 and U6 snRNAs do not (Fig. 1b). Dissociation of ribosomes into subunits by EDTA treatment resulted in a shift of HSUR 2, miRNAs, and β -Actin mRNA to lighter regions of the gradient, indicating that the presence of HSUR 2 in the polyribosomal region of the gradient is not due to association with a high-molecular weight, EDTA-insensitive particle (Fig. 1c). To test whether HSUR 2 associates with translationally active mRNAs, we treated cells with either puromycin or pactamycin, two drugs that inhibit protein synthesis by different mechanisms. As expected, treatment with either drug disassembled polyribosomes, increased levels of 80S ribosomes, and shifted miRNAs and mRNAs to a lighter region of the gradient (Fig. 1d). HSUR 2 exhibited the same shift, indicating that this viral snRNA associates with translating mRNAs (Fig. 1d).

To identify mRNAs directly bound by HSUR 2, we developed a method termed RNA-RNA interaction identification by crosslinking and capture (RICC), which combines *in vivo* psoralen crosslinking and specific HSUR 2 capture using biotinylated probe hybridization, with high-throughput sequencing (seq) to identify crosslinked RNAs (Fig. 2a). RNAs crosslinked to HSUR 2 were identified by comparison of pull downs from RNA samples prepared from HVS-transformed marmoset T cells, and pull downs from RNA samples prepared from the same cells in which HSUR 2 expression was knocked down before crosslinking by transient transfection with HSUR 2 antisense oligonucleotide (ASO)⁷ (Extended data Fig. 2b). RICC-seq identified 74 transcripts that were significantly (p-value < 0.05) enriched in HSUR 2 pull downs in at least three replicates (Supplementary Table 1). Most transcripts targeted by HSUR 2 correspond to cellular protein coding mRNAs, with a small subset of transcripts encoding viral proteins (Fig. 2b). Ingenuity pathway analyses of HSUR 2 targets, which include FAS¹⁷, the tumor suppressor retinoblastoma (RB1)¹⁸, and interferon- γ (IFN- γ)¹⁹ revealed enrichment of genes involved in regulation of cell cycle, apoptosis, and immune response (Fig. 2c and Supplementary

Table 2), all of which are relevant to HVS infection. These results provide confidence in RICC-seq as a method to detect HSUR 2 targets, and suggest a possible role for this viral snRNA in the regulation of these processes in virally transformed cells.

We hypothesized that HSUR 2-mediated recruitment of miR-142-3p and miR-16 would result in repression of HSUR 2 targets, primarily through mRNA destabilization²⁰. Transient knockdown of HSUR 2 in virally transformed marmoset T cells correlated with higher levels of HSUR 2 target mRNAs (Fig. 2d). No changes were observed for the same target mRNAs when a HSUR 2 ASO was transfected into marmoset T cells transformed with a mutant version of HVS that lacks HSURs 1 and 2^{7,21} (Extended data Fig. 3), arguing that HSUR 2 is specifically involved in regulating these target mRNAs. Ectopic expression of HSUR 2 together with other HSURs and HVS miRNAs in GSML cells, a B cell line derived from HVS natural host *Saimiri sciureus*, confirmed the repressive role of this viral snRNA on target mRNAs (Extended data Fig. 4). Transient transfection of a vector expressing only HSUR 2 sequence but not hvsa-miR-HSUR2 miRNAs (Extended data Fig. 4b) showed that HSUR 2 expression is necessary and sufficient to repress target mRNAs (Fig. 2e). Moreover, no changes were observed when cells were transfected with a vector carrying only HSUR 2 transcriptional signals, suggesting that the downregulation of HSUR 2 target mRNAs is not due to titration of transcription factors. Similar results were obtained when HSUR 2 was expressed in the human B cell line BJAB (Extended data Fig. 5). Furthermore, we analyzed the levels of proteins whose mRNAs are targeted by HSUR 2 in marmoset T cells newly transformed with either wild-type HVS (cj137-WT cells), or a mutant HVS lacking only HSUR 2 (cj137- HSUR2 cells) (Extended data Fig. 6). Absence of HSUR 2 correlated with upregulation of several proteins encoded by HSUR 2 target mRNAs (Fig. 2f). These results indicate that RICC-seq successfully identified mRNAs repressed by HSUR 2.

HSUR 2 associates with Ago proteins⁷. Transient knockdown of HSUR 2 resulted in lower association of HSUR 2 target mRNAs with Ago proteins, as revealed by Ago immunoprecipitation experiments (Fig. 3a), supporting the hypothesis that HSUR 2 interaction with target mRNAs results in recruitment of Ago proteins specifically to these transcripts. Inhibition of miR-142-3p and miR-16 activity with locked nucleic acid (LNA) inhibitors did not affect the expression of HSUR 2 (Extended data Fig. 7a) nor its ability to base-pair with mRNAs (Extended data Fig. 7b), but showed that miR-142-3p activity is required for HSUR 2-mediated repression of all tested target mRNAs, whereas miR-16 expression is required for repression of a subset of HSUR 2 targets (Fig. 3b). Mutations in miRNA-binding sites present in HSUR 2 did not affect the expression of this viral Sm-class RNA (Extended data Fig. 8a) but affected its ability to destabilize target mRNAs (Fig. 3c and Extended data Fig. 8b). Direct interaction between miR-142-3p and HSUR 2 is required for HSUR 2-mediated repression of all tested target mRNAs since cells transfected with a mutant version of HSUR 2 unable to bind this miRNA (HSUR2 142-3p; Fig. 3c and Extended data Fig. 8b) have levels of target mRNAs comparable to cells transfected with an empty vector. Similarly, direct interaction between HSUR 2 and miR-16 is required for HSUR 2-mediated repression of some (e.g., RB1) but not all HSUR 2 targets (HSUR2 16; Fig. 3b and Extended data Fig. 8b). Expression of a mutant version of HSUR 2 with U→G substitutions, which are expected to interfere with ARE function¹¹, in the two ARE-like sequences had a weak, albeit not statistically significant effect on the levels of some of

HSUR 2 target mRNAs (i.e. *RASA2* and *DDX20* mRNAs, Extended data Fig. 8b). This, however, does not rule out the possibility that the ARE sequences present in HSUR 2 could have an important role in the regulation of the stability and/or translation of some of the mRNAs targeted by HSUR 2.

The ability of HSUR2 142-3p and HSUR2 16 to repress target mRNAs was restored by co-transfection of mutant versions of miR-142-3p and miR-16 with seed regions modified to have complementarity to the corresponding mutant HSUR 2 (miR-142-3p_{mut} and miR-16_{mut}; Fig. 3d and 3e; Extended data Fig. 9). These results confirm that interactions between HSUR 2 and these two cellular miRNAs are necessary for HSUR 2-mediated mRNA repression. These results also suggest that HSUR 2-mediated repression of target mRNAs is not the consequence of HSUR 2 inhibiting miR-142-3p and miR-16 activities, and favor a model in which HSUR 2 recruits these miRNAs to target mRNAs.

HSUR 2-mediated mRNA repression has an important role in viral modulation of apoptosis. Staining of cj137-WT and cj137- HSUR2 cells with Annexin V/propidium iodide (PI) showed that absence of HSUR 2 correlates with a higher percentage of cells undergoing apoptotic cell death (Fig. 4a). Marmoset T cells lacking HSUR 2 also showed higher activity of the key effector caspase-3/7 when compared to cells expressing this viral snRNA, whereas both cell lines showed similar activity of the initiator caspase-8 (Fig. 4b). Transient expression of only HSUR 2 in BJAB cells resulted in decreased caspase-3/7 activity upon FAS-induced (Fig. 4c) as well as tumor necrosis factor alpha (TNF- α)-induced apoptosis (Extended data Fig. 10a), confirming a role for this viral snRNA in the regulation of apoptosis. To test if HSUR 2-mediated mRNA repression is necessary for regulation of apoptosis, we expressed mutant versions of HSUR 2 that are not able to bind miR-142-3p and/or miR-16 in BJAB cells and measured caspase-3/7 activity upon induction of apoptosis. Interaction of HSUR 2 with both miRNAs is required to confer resistance to FAS-induced (Fig. 4c) and TNF- α -induced (Extended data Fig. 10a) apoptosis. Furthermore, co-transfection of miR-142-3p_{mut} or miR-16_{mut} restored the ability of mutant versions of HSUR 2 to confer resistance to apoptosis (Fig. 4d and Extended data Fig. 10b). These results suggest that HSUR 2 restricts apoptosis by recruiting host miR-142-3p and miR-16 to target mRNAs encoding key apoptotic factors, rather than by affecting the activity of these miRNAs.

We have demonstrated that HSUR 2 snRNA interacts with specific mRNAs in virally transformed T cells (Fig. 1). Psoralen-dependent crosslinking indicates that these interactions occur via base-pairing (Fig. 1a). Expression of HSUR 2 results in destabilization of target mRNAs (Fig. 2). Presumably, base-pairing with target mRNAs is required for HSUR 2-mediated repression. Further investigation will be required to elucidate the mechanism by which HSUR 2 is recruited to target mRNAs. HSUR 2-mediated mRNA destabilization requires binding of this viral Sm-class RNA to miR-142-3p and, in some cases, miR-16 (Fig. 3), suggesting that HSUR 2 functions by recruiting these miRNAs to target mRNAs (Fig. 4e). HSUR 2-mediated tethering of miRNAs to target mRNAs results in recruitment of Ago proteins (Fig. 3a), which promote translational repression/degradation via deadenylation of the target mRNA²². Since HSUR 2 is not polyadenylated, association with Ago proteins does not affect its expression⁷ (Extended data Figs. 7a and 8a). HSUR 2 is

more abundant than target mRNAs, but less abundant than miR-142-3p and miR-16 (Supplementary Table 3). This stoichiometry is probably important to ensure effective miR-142-3p- and miR-16-dependent, HSUR 2-mediated mRNA repression²³. Disruption of HSUR 2 ARE-like sequences did not have a statistically significant effect on target mRNA destabilization (Fig. 3c and Extended data Fig. 8b). These sequences, as well as the recruited miRNAs, could possibly modulate the translation of target mRNAs²⁴.

Our results uncover a novel role for a viral Sm-class RNAs in post pre-mRNA-processing regulation of gene expression. Interestingly, this function is reminiscent of those of the structurally related bacterial Hfq-associated regulatory RNAs that regulate stability and translation of target mRNAs²⁵. Since viruses often co-opt factors from their hosts, it is therefore conceivable that similar ncRNAs exist in host cells to regulate gene expression in a similar fashion²⁶. Further investigation will be required to probe these hypotheses.

Methods

Plasmids

For selection of transfected cells based on expression of the green fluorescent protein (GFP) by FACS, a fragment containing the human cytomegalovirus (CMV) intermediate early enhancer/promoter, the GFP coding region, and the polyadenylation signal of the simian virus 40 (SV40) was generated by PCR using pEGFP-C2 (Clontech) as template, and inserted between the *KpnI* and *XhoI* sites of pBluescript II SK+ (Stratagene) to generate the plasmid pBS-GFP (referred to as “Empty vector” in Figs. 2e and 3, and Extended data Figs. 4d, 5, 8, and 9). For expression of HSURs and HVS miRNAs, a genomic fragment including positions +1444 and +3504 of the genome of HVS-A11 (GenBank: X64346.1) was inserted between the *SaI* and *BamHI* sites of pBS-GFP to generate the plasmid pBS-GFP-H1-5 for expression of HSURs 1, 2, and 5 together with hvsa-miR-HSUR2 and hvsa-miR-HSUR5 miRNAs (“HSURs1-5”, Extended data Fig. 4a). A genomic fragment including positions +4613 and +6945 was inserted between the *SaI* and *BamHI* sites of pBS-GFP to generate the plasmid pBS-GFP-H3-7 for expression of HSURs 3, 4, 6, and 7 together with hvsa-miR-HSUR4 miRNAs (“HSURs3-7”, Extended data Fig. 4a). For expression of HSUR 2 only and not hvsa-miR-HSUR2 miRNAs, a fragment of HVS A11 genome including positions +2344 to +2726 was inserted between the *EcoRI* and *BamHI* sites of pBS-GFP to generate the plasmid pBS-GFP-HSUR2 (Extended data Fig. 4b). The plasmid pBS-GFP- HSUR2 (provided by Soledad Camolotto) containing only HSUR 2 promoter and enhancer sequences, but not HSUR 2 sequence (Extended data Fig. 4b) was generated by insertion of the HVS A11 genomic fragment including positions +2486 to +2726 between the *EcoRI* and *BamHI* sites of pBS-GFP. Mutant versions of HSUR 2 (plasmids pBS-HSUR2 142-3p, pBS-HSUR2 16, pBS-HSUR2 ARE, and pBS-HSUR2 142-3p/16) were obtained by site-directed mutagenesis using a QuickChange Site-Directed Mutagenesis Kit (Stratagene) and confirmed by sequencing.

Recombinant herpesvirus saimiri

Bacmid constructs HVS-BAC-GFP-WT and HVS-BAC-GFP- HSUR2 were achieved by *en passant* mutagenesis^{27,28}. First, we generated a plasmid that contains the first ~ 7 kilobases

(kb) of HVS A11 genome by insertion of a HVS A11 genomic fragment generated by overlapping PCR with two fragments: one fragment including positions +22 to +2354 of HVS A11 genome, and a second fragment including positions +2547 to +7249. The generated fragment, which lacks both HSUR 2 and HSUR 2 promoter sequence required for expression of *hvs*a-miR-HSUR2 miRNAs²⁹, was inserted between the *SaI* and *Bam*HI sites of pBluescript II SK+ to generate the plasmid pBS-7.4 HSUR2. A unique *Nco*I site was generated by site-directed mutagenesis in both pBS-7.4 HSUR2 and pBS-7.4²⁹ at a position corresponding to +3664 of HVS A11 genome to generate the plasmids pBS-7.4 HSUR2/*Nco* and pBS-7.4/*Nco*. A PCR amplifcon containing the kanamycin resistance gene, the *I-Sce*I restriction site, and a 50 bp duplication of HVS A11 genomic sequence was generated using the pEPkanS2 vector²⁷ (kindly provided by Karsten Tischer) and inserted into the *Nco*I site of both pBS-7.4 HSUR2/*Nco* and pBS-7.4/*Nco* to generate the plasmids pBS-7.4 HSUR2/*Nco*/Kan and pBS-7.4/*Nco*/Kan, respectively. PCR-amplified fragments encompassing positions +65 to +4637 of the HVS A11 genome were generated using pBS-7.4 HSUR2/*Nco*/Kan and pBS-7.4/*Nco*/Kan as template (Extended data Fig. 6a), and recombined into the bacmid HVS-BAC-GFP³⁰ (kindly provided by Adrian Whitehouse) in GS1783 cells (a generous gift from Greg Smith) as previously described²⁸. The bacmid HVS-BAC-GFP carries the *Sac*I deletion³¹ that removes part of the saimiri transforming protein-A (STP-A) coding region, together with the genes encoding HSURs 1, 2, and 5, *hvs*a-miR-HSUR2 and *hvs*a-miR-HSUR5 miRNAs, and part of vDHFR (Extended data Fig. 6a). Viruses generated with HVS-BAC-GFP are therefore unable to transform cells^{30,31}. Recombinants were selected on chloramphenicol/kanamycin plates. The scarless removal of the kanamycin cassette was achieved by induction of *I-Sce*I expression during the second Red-recombination step through arabinose induction, generating the bacmids HVS-BAC-GFP-WT and HVS-BAC-GFP- HSUR2. Positive clones were screened by PCR, and analyzed by restriction endonuclease (Extended data Fig. 6b) and sequencing.

Virus cultures, marmoset T cell immortalization, and cell lines

Recombinant viruses were generated by transfection of bacmids HVS-BAC-GFP-WT and HVS-BAC-GFP- HSUR2 into owl monkey kidney cells (OMK; ATCC, OMK-637-69) using Lipofectamine 2000 (ThermoFisher Scientific) following the manufacturer's instructions. Complete cytopathic effects (CPE) were observed around 14 days after transfection. High titer virus stocks were generated by infecting 60–80% confluent OMK cells in T-75 cm² flasks with low multiplicity of infection (MOI 0.1). Virus titers were estimated by the limited dilution protocol as described³². *In vitro* immortalization of marmoset T cells without IL-2 was performed following established protocols³³. Preservative-free heparinized marmoset blood samples from three different animals (cj137, cj290, and cj323) were obtained from PrimateBiologicals (<http://primatebiologicals.com/>). Although all samples were successfully immortalized, only cell lines derived from animal cj137 (cj137-WT and cj137- HSUR2; Figs. 2f, 4a, and 4b, and Extended data Fig. 6) continued to grow after 6 months of cultivation. Cell lines cj137-WT and cj137- HSUR2, as well as marmoset T cell lines derived from a single marmoset that were transformed in parallel with either wild-type HVS A11 or HVS A11 2a²¹ deleted for the HSUR 1 and 2 genes¹⁰ (cj319-WT and cj319- 2a; Fig. 1 and 2d, and Extended data Figs. 1 and 3) were grown in RPMI 1640 medium (ThermoFisher Scientific) supplemented with 20% fetal

bovine serum (FBS), 100 U/mL of penicillin, 100 µg/mL of streptomycin, 2 mM glutamax (ThermoFisher Scientific), 1 mM sodium pyruvate (ThermoFisher Scientific) and 0.001% β-mercaptoethanol (Sigma Aldrich). Epstein Barr Virus (EBV)-transformed B-lymphoblast GSML cells³⁴ (ATCC-CRL-2699) derived from the squirrel monkey *Saimiri sciureus* and human Burkitt's lymphoma BJAB cells (a kind gift from Joan Steitz) were not authenticated and were grown in RPMI 1640 medium supplemented with 10 % FBS, penicillin, streptomycin, sodium pyruvate and glutamax. BJAB cells were used for apoptosis assays (Fig. 4 and Extended data Fig. 10) since they express miR-142-3p and are not transformed by EBV. All cell lines were routinely tested for presence of mycoplasma.

Antibodies

Polyclonal antibodies to STAG1 (Catalog # LS-C161537, Lot # 49409) and NGDN (Catalog # 16524-1-AP) were purchased from Life Span Biosciences and Proteintech Group, respectively. Antibodies to DDX5 (Catalog # 4387S), Rock2 (Catalog # 8236S), Rac1/2/3 (Catalog # 2465S), N-Wasp (Catalog # 4848S), Fas (Catalog # 8023S) and retinoblastoma (Catalog # 9309S) were from Cell Signaling Technology. Polyclonal antibodies to GRLF1 (Catalog # A301-736A-M) were obtained from Bethyl Laboratories whereas mouse monoclonal antibody to α-tubulin (Catalog # SC-23948, Lot # K0812) was obtained from Santa Cruz Biotechnology. Monoclonal antibodies to Argonaute proteins (PAN-Ago, clone 2A8) were a gift from Zissimos Mourelatos.

Polysome gradients

Soluble extracts from cj319-WT cells left untreated or treated for 16–18 hours with 200 µg/mL of puromycin (Invivogen) or 2.5 µg/mL of pactamycin (Sigma Aldrich) were analyzed on 12-mL linear 15–50% sucrose gradients prepared in polysome buffer (20 mM Tris-HCl, pH 7.5, 150 mM KCl, 10 mM MgCl₂ and 100 µg/mL of cycloheximide (Sigma Aldrich) and equilibrated at 4°C for 3 hours. Briefly, cells were harvested, centrifuged and suspended in 20 mL of complete RPMI 1640 media plus 100 µg/mL of cycloheximide. Cells were incubated at 37°C/5 % CO₂ for 25 min and centrifuged. Cells were suspended in 4 volumes of hypotonic lysis buffer (5 mM Tris-HCl, pH 7.5, 1.5 mM KCl, 10 mM MgCl₂, 5 mM DTT, 0.5% Triton X-100, 0.5% sodium deoxycholate, 10 µg/mL digitonin, 10 mM ribonucleoside vanadyl complex (New England Biolabs) preheated at 65°C and 100 µg/mL of cycloheximide). Cells were vortexed and passed three times through a gauge 25 needle and then three times through a gauge 27½ needle. The extracts were clarified at 17,000 × *g* for 10 min at 4°C and heparin (ThermoFisher Scientific) was added to a final concentration of 1 mg/mL. Where indicated EDTA was added to the clarified extract to a final concentration of 50 mM and incubated on ice for 20 min. Three hundred microliter samples from the extracts were layered atop duplicate sucrose gradients and centrifuged at 150,000 × *g* on a Beckman SW41 rotor for 2 hours at 4°C. The sedimentation profile of one of the gradients was determined using a 65% sucrose pump at a constant flow rate of 1.5 mL/min linked to an ISCO UA-6 254-nm ultraviolet detector equipped with a chart recorder set at 60 cm/h and 1 AU full scale. The second gradient was fractionated (0.5 mL per fraction) and the RNA was purified by acid phenol-chloroform extraction and 3 M sodium acetate-ethanol precipitation, and analyzed by Northern blot.

Northern blotting

RNA from each fraction of the sucrose gradients was suspended in 20 μ L of 1X DNase reaction mixture: 2 μ L of 10X DNase reaction buffer, 0.4 units of RNase-free DNase I (New England Biolabs) and 17.8 μ L of H₂O, and incubated at 37°C for 30 min. The volume of each sample was taken to 0.5 mL with H₂O and the RNA was re-purified by acid phenol-chloroform extraction and sodium acetate-ethanol precipitation. RNA samples were then suspended in 20 μ L of 2 mM Tris-HCl, pH 7.5, 8 M urea and 20 mM EDTA and heated at 65°C before loading onto a 14% denaturing polyacrylamide gel. Gels were run at 10 W in 1X TBE and transferred onto a Zeta-Probe® nylon membrane (Bio-Rad) for 30 min at 1 A using a semi-dry blotting unit (Fisher Biotech). The membranes were pre-hybridized at 42°C in ExpressHyb hybridization solution (Clontech) for at least one hour. Hybridization of ³²P-labeled probes to HSUR 2, U2 and U6 was performed in ExpressHyb solution overnight at 42°C. Hybridization of ³²P-labeled probes to miR-142-3p, miR-16 and miR-21 was performed overnight at 30°C. Blots were washed once with 2X saline sodium citrate (SSC) buffer, 0.1% SDS for 15 min at room temperature, followed by either a wash with 1X SSC, 0.1% SDS for 15 min at room temperature (miR-142-3p, miR-16, and miR-21 probes) or a wash with 0.5X SSC, 0.1% SDS for 15 min at room temperature (HSUR 2, U2, and U6 probes). Membranes were wrapped in saran wrap and exposed to a phosphorImager screen (GE Healthcare). To detect β -actin mRNA in the polysome gradient fractions, RNA was suspended in 25 μ L of 20 mM 3-(N-morpholino)propanesulfonic acid (MOPS), pH 7.0, 15% formaldehyde, 50% deionized formamide and 20 mM EDTA, heated at 95°C for 15 min, placed on ice and loaded onto 1.2% agarose-formaldehyde gels. The gels were run at 100 V in 20 mM MOPS, pH 7.0, 2.6% formaldehyde and transferred overnight onto nylon membranes by capillarity. The membranes were stained with 0.02% methylene blue in 0.4 M sodium acetate and 0.4 M acetic acid and destained in H₂O. Blots were prehybridized in ExpressHyb solution at 42°C for 4 hours and hybridized with ³²P-labeled probe to β -actin mRNA overnight at 80°C. The membranes were washed with 2X SSC, 0.1% SDS for 15 min at room temperature followed by a wash with 0.5X SSC, 0.1% SDS for 15 min at room temperature. Membranes were wrapped in saran wrap and exposed to a phosphorImager screen.

Probe radiolabeling

Radiolabeled probes were prepared in 20 μ L reactions containing 10 pmoles of antisense oligonucleotide (ASO) to HSUR 2, U2, U6, miR-142-3p, miR-16 or miR-21, 10 units of T4 polynucleotide kinase (New England Biolabs) and 151.5 μ Ci of [γ -³²P]ATP (6000 Ci/mmol, PerkinElmer) and incubated at 37°C for one hour. Unincorporated isotope was removed by centrifugation using Mini Quick® G-25 gel filtration columns following the manufacturer's protocol (Roche). Radiolabeled probes were eluted in a volume of 50 μ L of H₂O and 10 μ L were used in each hybridization experiment. A β -actin probe was labeled using 50 μ Ci of [α -³²P]dCTP and Klenow DNA polymerase for 15 min using the DecaPrime II kit according to the manufacturer's instructions (Ambion). A 10- μ L aliquot of the radiolabeled probe was taken to a volume of 100 μ L with 10 mM EDTA, pH 8.0 and incubated at 95°C for 10 min. The labeled probe was then added directly without further purification to the prehybridized membranes.

Immunoblotting

Marmoset cj137-WT and cj137- HSUR2 cells were harvested and stained with 100 ng/mL of 2-(4-aminophenyl)-1H-indole-6-carboxamide (DAPI) and FACS-sorted in a BD FACSARIA-II cell sorter (BD Biosciences) equipped with a UV laser at the University of Utah Flow Cytometry Core. Live, DAPI-negative cells were collected and total cell lysates were prepared in Glo Lysis Buffer™ (Promega) containing Complete™ protease inhibitors (Roche), 10 mM *N*-ethylmaleimide (Sigma Aldrich) and 1 mM phenylmethylsulfonyl fluoride (Fluka BioChemika). Cj319-WT and cj319- 2a cells were harvested, washed with PBS and directly lysed for analysis as described above. The protein concentration of the lysates was determined with the Coomassie Plus™ Protein Assay reagent (ThermoFisher Scientific). Lysates were boiled with 1X SDS-PAGE sample buffer and 15 or 20 µg of total protein were separated on 10% high Tris polyacrylamide gels (0.75 M Tris base) using high Tris running buffer (50 mM Tris base, 193 mM glycine, 0.1% SDS). Proteins were transferred onto nitrocellulose membranes, probed with the indicated antibodies and visualized by enhanced chemiluminescence according to standard protocols.

Transfections

Ten million cj319-WT or cj319- 2a cells were nucleofected with Control (complementary to GFP coding sequence) or HSUR 2 chimeric ASO (see **RICC-seq** described below) (Integrated DNA Technologies) as described above using Amaxa's human T-cell kit (Lonza) and program X-001 in a nucleofector™ 2b device. Twenty-one hours after transfection the cells were harvested, washed with PBS and stored in TRIzol (ThermoFisher Scientific) at -80°C until used. GSML and BJAB cells were nucleofected using 5×10^6 and three million cells, respectively, per nucleofection with Amaxa's kit V (Lonza) and program T-020, and either 3.4 µg of pBS-GFP, 1 µg of vector encoding pBS-GFP-HSUR 2 (and mutant variants of HSUR 2), or 1 µg of pBS-GFP HSUR2. In experiments in which GSML or BJAB cells were co-transfected with miR-142-3p_{mut} or miR-16_{mut} (Integrated DNA Technologies), 50 pmoles of either mutant miRNA or 50 pmoles of control siRNA-A (Santa Cruz Biotechnology) were added to the nucleofection reaction. To test the effect of locked nucleic acid (LNA) miRNA inhibitors on HSUR 2-mediated repression of HSUR 2 targets, pBS-GFP or pBS-GFP-HSUR2 plasmid was co-transfected with either 100 pmoles of control LNA (miRCURY LNA™ microRNA Inhibitor Negative Control A), or 50 pmoles of either miRCURY LNA™ miRNA inhibitor against hsa-miR-142-3p (Exiqon) or miRCURY LNA™ miRNA inhibitor against hsa-miR-16 (Exiqon) plus 50 pmoles of control LNA, or 50 pmoles each of both miR-142-3p and miR-16 LNA inhibitors. Transfections of GSML cells with pBS-GFP-H1-5 and pBS-GFP-H3-7 were performed with 5 µg of plasmid plus 3.4 µg of pBS-GFP, or 5 µg each of both pBS-GFP-H1-5 and pBS-GFP-H3-7. In all the experiments described above, transfected GSML or BJAB cells were grown for 18–21 hours after nucleofection and FACS-sorted on a BD FACSARIA-II cell sorter equipped with a 488 nm laser (525/50 filter set) for GFP-positive cells. Cells that were collected expressed a mid-range level of GFP (arbitrary fluorescence of 10^3 – 10^5 within the GFP window; initial experiments showed that cells expressing GFP beyond 10^5 were necrotic as revealed by DAPI staining and were therefore discarded and excluded from further analysis).

Immediately after sorting cells were centrifuged, suspended in 100 µl of culture medium and stored in TRIzol at –80°C until used.

Quantitative RT-PCR

Total RNA was isolated from cj319-WT, cj319- 2a, GSML, or BJAB cells with TRIzol according to the manufacturer's instructions. The TRIzol-purified total RNA was suspended in 85 µl of water and 10 µl of 10X DNase-I buffer and treated with 10 units of RNase-free DNase I (New England Biolabs) at 37°C for 30 min and repurified with acid phenol-chloroform extraction followed by sodium acetate-ethanol precipitation. cDNA was synthesized in 20 µl reactions using the High Capacity cDNA Reverse Transcription Kit with MultiScribe Reverse Transcriptase (Applied Biosystems) using 600–900 ng of DNase-I-treated total RNA and random primers. Subsequently, real-time PCR was performed in 8 µl reactions using primers (Supplementary Table 4) at 0.5 µM, cDNA diluted 1:3 to 1:7.5 (depending upon the input of total RNA used in the cDNA synthesis reaction as described above) and KAPA SYBR green (KAPA Biosystems) in a Roche 480 LightCycler (95°C for 5 min, 1 cycle; 95°C for 10 sec, 60°C for 10 sec and 72°C for 10 sec, 55 cycles followed by a melting curve of the reaction product from 65°C to 97°C with a ramp rate of 0.11°C/sec). qPCR primers were designed using Primer3Plus and tested using cDNA prepared from 900 ng of DNase-treated total RNA and diluted from 1:5 to 1:5000. All reactions were performed at least four times in each independent experiment. C_T values were determined using the “Abs quant/2nd derivative max” function of the LightCycler. Relative mRNA levels of HSUR 2 targets were calculated using the 2^{-C_T} method³⁵ using the following equation:

$$\text{Relative mRNA level} = \frac{2^{-\Delta C_T} \text{ HSUR2 target in treated sample (i. e. , plus HSUR2, etc.)}}{2^{-\Delta C_T} \text{ HSUR2 target in control sample (i. e. , plus Empty vector or } \Delta \text{HSUR2)}}$$

For normalization the C_T values of 18S ribosomal RNA were subtracted from the C_T values of HSUR2 targets to calculate the C_T .

Estimation of the number of molecules of HSUR 2 and HSUR 2 target mRNAs in WT and HSUR2 marmoset T cells

Sequences of select HSUR 2 targets were amplified from marmoset T-cell cDNA by PCR using *Taq* polymerase and pairs of primers listed in Supplementary Table 4, and introduced into the pCR2.1-TOPO vector by TA-cloning (Invitrogen). Plasmids encoding sequences of HSUR 2 targets were purified, quantitated and used to prepare 1:10 serial dilutions from 100 pg/µl to 100 ag/µl. Two microliter samples of each dilution were then used in real-time PCR reactions using KAPA SYBR green as described above to generate standard curves (Log (number of molecules) vs C_T) for each target. A standard curve for HSUR 2 was also generated using 1:10 serial dilutions of pBS-HSUR2 miR as template. Total RNA from 4×10^6 WT or 3.05×10^6 HSUR 2 marmoset T-cells was isolated with TRIzol, and 900 ng were used to synthesize cDNA as described above. The resulting cDNAs were diluted 1:5 for analysis of HSUR 2 targets, and diluted 1:1000 for analysis of HSUR 2. Subsequently, qPCR was performed as described above. The resulting C_T values were used to estimate the

number of molecules for HSUR 2 and for HSUR 2 targets using the previously generated standard curves.

Analysis of mRNAs co-immunoprecipitated with Ago proteins

Immunoprecipitation—Thirty to forty million cj319-WT cells were transfected with Control or HSUR 2 ASO as described above and used to prepare whole cell extracts in 0.35–0.4 ml of NET-2 buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.05% Nonidet-40) containing cOmplete™ protease inhibitors, 1 mM PMSF and 5 µl of RNaseOUT™ (ThermoFisher Scientific). Extracts were sonicated on ice three times for 10 sec each. Lysates were cleared by centrifugation at $16,000 \times g$ for 10 min at 4°C. Aliquots equal to 10% of each lysate were saved and stored immediately in 1 ml of TRIzol. Rabbit anti-mouse IgG (ThermoFisher Scientific) was immobilized on Protein A Sepharose 4 Fast Flow beads (GE Healthcare) in bulk overnight at 4°C and aliquoted into corresponding tubes before the last wash to guarantee that all samples were incubated with the same amount of antibody. The remaining 90% fraction of each lysate was incubated with 5 µl of anti-PAN-Ago antibodies -or normal mouse serum (Sigma Aldrich)- and 30 µl of rabbit anti-mouse IgG with continuous rotation for 4 hours at 4°C. Samples were washed four times with NET-2 buffer containing protease inhibitors and the RNA immunoprecipitation “RIP” fractions were collected by centrifugation and stored immediately in TRIzol at –80°C.

RNA purification and cDNA synthesis—Total RNA in TRIzol from Input and RIP fractions was isolated according to the manufacturer’s instructions using 20 µg of glycogen during the precipitation step. Total RNA was resuspended in 15 µl of water and used for cDNA synthesis as described above except that 1.5 µl of each Input fraction (1% of the total) and 14.2 µl of each RIP fraction were used per 20-µl reaction.

Quantitative PCR of immunoprecipitated mRNAs—Real-time PCR of HSUR 2 targets was performed as described above using cDNAs from Input and RIP fractions diluted 1:5. The C_T values of HSUR 2 targets were normalized to the Input RNA fraction C_T values according to the following equation:

$$\text{i. } \Delta C_T [\text{normalized RIP}] = (C_T [\text{RIP}] - (C_T [\text{Input}] - \log_2(\text{Input Dilution Factor})))$$

Since 1% of each Input fraction was used to synthesize cDNA: $\log_2 100 = 6.644$

The normalized RIP fraction C_T value was then adjusted for the normalized background (normal mouse serum reaction (NS)) fraction C_T value as follows:

$$\text{ii. } \Delta \Delta C_T [\text{RIP/NS}] = \Delta C_T [\text{normalized RIP}] - \Delta C_T [\text{normalized NS}]$$

The fold-enrichment of each HSUR 2 target in the RIP fraction above the sample specific background was then calculated with the following equation:

$$\text{iii. } \text{Fold Enrichment} = 2^{(-\Delta \Delta C_T [\text{RIP/NS}])}$$

$$\text{iv. } \text{Fold Enrichment [Control ASO/HSUR 2 ASO]} = \frac{2^{(-\Delta \Delta C_T (\text{RIP/NS}))_{\text{Control ASO}}}}{2^{(-\Delta \Delta C_T (\text{RIP/NS}))_{\text{HSUR 2 ASO}}}}$$

Apoptosis assays

Growing cultures of cj137-WT and cj137- HSUR 2 cells were harvested and layered atop 10-mL cushions of Histopaque®-1077 (Sigma Aldrich) to remove cell debris. The cells were centrifuged at $400 \times g$ for 30 min at room temperature, and cells at the interface were collected, washed, suspended in complete medium and counted. Cells were seeded at a density of 500,000 cells/mL and grown for two weeks. Cells were then harvested, washed in ice-cold PBS, centrifuged and suspended at a density of 10^6 cells/mL in 100 μ L of annexin-binding buffer (10 mM HEPES, pH 7.4, 140 mM NaCl and 2.5 mM CaCl_2). Cells were stained for 15 min at room temperature with Alexa Fluor® 647 annexin V conjugate (1:20 dilution, Life Technologies) and 1 μ g/mL of propidium iodide (Life Technologies). Samples were diluted with 400 μ L of annexin-binding buffer and placed on ice. Data were acquired on a BD FACSCanto (BD Biosciences) flow cytometer using the 488 nm (685/40 filter set) and 640 nm (670/30 filter set) lasers. Alternatively, cj137-WT and cj137- HSUR2 cells were stained with 100 ng/mL of DAPI, and live, DAPI-negative cells were FACS-sorted directly into a 96-well plate at a density of 20,000 cells per well. Caspase activity was then measured with the Caspase-Glo® 3/7 and Caspase-Glo® 8 reagents according to the manufacturer's instructions (Promega). Caspase 3/7 activity was also measured in BJAB cells transiently expressing either HSUR 2 or HSUR 2 variants (Fig. 4 and Extended data Fig. 10). To this end, BJAB cells were transiently transfected with either pBS-GFP-

HSUR2, pBS-GFP-HSUR2 142-3p, pBS-GFP-HSUR2 16, or pBS-GFP-HSUR2 142-3p/16. GFP-positive cells were FACS-sorted and suspended in culture medium to a density of 400,000 cells/mL, and 100 μ L were seeded per well of a 96-well plate. The cells were then incubated with 100 ng/mL of recombinant soluble Fas ligand (PeproTech) or 100 ng/mL of TNF- α (Sigma Aldrich) for 19 hours at 37°C. Caspase 3/7 activity was then assayed as described above.

RICC-seq

***In vivo* RNA-RNA crosslinking**—Two sets of three independent samples of 6×10^7 cj319 cells each were treated with chimeric ASOs (the oligonucleotides are 20-nt long, contain backbone phosphorothioate linkages to increase stability, and have five nucleotides on each end substituted with 2'-O-methoxyethyl ribonucleotides^{7,36}) directed to GFP (5'-UCACCTTCACCCTCTCCACU-3') (control HSUR 2-positive sample) or an ASO directed to HSUR 2 (5'-AAGCGATACCTCGTGUGUGA-3') which specifically targets HSUR 2 for degradation (HSUR 2-negative sample)⁷ (Extended data Fig. 2). Cells were transfected (10^7 cells per nucleofection) with 1 nmol of ASO by applying three pulses of 1700 V for 10 milliseconds each (Neon System, ThermoFisher Scientific). Cells were then plated at a density of 1×10^6 cells/mL, grown at 37°C/5 % CO_2 for 24 hours and harvested. Cells from each sample were pooled, washed with phosphate-buffered saline (PBS) and suspended in 1 mL of PBS containing 200 μ g/mL 4'-aminomethyl-4,5',8-trimethylpsoralen (AMT, Cayman Chemical), crosslinked as already described⁷, collected, and fractionated in 100- μ L samples. One hundred microliters of 6 M guanidinium hydrochloride was added to each sample followed by 20 μ L of a 20 mg/mL solution of RNase-free proteinase K (Ambion) and 5 μ L of 20% sodium dodecyl sulfate (SDS). Samples were incubated at 65°C for one hour. One milliliter of TRIzol was added to each sample and then stored at -80°C.

PolyA⁺ RNA purification—Total RNA was purified from control HSUR 2-positive and HSUR 2-negative samples in TRIzol according to the manufacturer's protocol. PolyA⁺ RNA was isolated using oligo d(T)25 magnetic beads (New England Biolabs). Briefly, purified total RNA was suspended in 600 μ L of H₂O and heated to 65°C for 5 min. One volume of 2X binding buffer (40 mM Tris-HCl, pH 7.5, 1 M NaCl, 1% SDS, 2 mM EDTA, 10 mM DTT) was added to each sample and mixed with 250 μ L of oligo d(T)25 magnetic beads. Samples were incubated with continuous rotation for 30 min at room temperature. The beads were washed twice for 1 min with 1 mL of wash buffer 1 (20 mM Tris-HCl, pH 7.5, 500 mM NaCl, 0.1% SDS, 1 mM EDTA, 5 mM DTT) and then once with 1 mL of wash buffer 2 (20 mM Tris-HCl, pH 7.5, 500 mM NaCl, 1 mM EDTA). The beads were then incubated with 1 mL of low salt buffer (20 mM Tris-HCl, pH 7.5, 200 mM NaCl, 1 mM EDTA) for 1 min at room temperature. PolyA⁺ RNA was eluted from the beads with 500 μ L of elution buffer (20 mM Tris-HCl, pH 7.5, 1 mM EDTA) at 50°C for 5 min with continuous agitation. PolyA⁺ RNA was precipitated by adding 50 μ L of 3 M sodium acetate, pH 5.2, 5 μ L of a 10 mg/mL solution of glycogen and 1 mL of 100% ethanol and incubated overnight at –20°C.

Capture of HSUR 2 with biotinylated ASO—PolyA⁺ from HSUR 2-positive or HSUR 2-negative samples was suspended in 500 μ L of H₂O, heated to 95 °C for 3 min and placed on ice. Five hundred pmoles of biotinylated ASO to HSUR 2 (5'-GGTTTTTAAATATGTACACCC-3'-Bio) were added to each sample. The samples were incubated at 65°C for 5 min and 500 μ L of 2X binding buffer (20 mM Tris-HCl, pH 7.5, 1 M NaCl, 2 mM EDTA, 0.1% SDS) heated at 65°C was added to each sample. One hundred microliters from each sample were saved as "PolyA⁺ input" samples and stored in 1 mL of TRIzol at –80°C until used. The remaining 900 μ L from each sample were incubated 4 hours at room temperature with continuous rotation. After annealing was completed, 125 μ L of MPG® streptavidin magnetic beads (PureBiotech) were added to each sample and incubated overnight at 4°C. The beads were washed twice with 1X binding buffer (10 mM Tris-HCl, pH 7.5, 500 mM NaCl, 1 mM EDTA, 0.05% SDS) and twice with 1X wash buffer (10 mM Tris-HCl, pH 7.5, 200 mM NaCl, 1 mM EDTA, 0.05% SDS). Bound RNA was recovered by adding 1 mL of TRIzol to the beads. RNA was purified from both the input and HSUR 2 ASO-treated samples following the manufacturer's protocol.

Library preparation and high-throughput sequencing—Purified RNA samples were suspended in 25 μ L of H₂O and placed on a glass plate covered with parafilm on ice. The AMT crosslinks were reversed by directly irradiating the samples at 254 nm for 10 min. The samples were collected and taken to a volume of 300 μ L with H₂O. RNA was precipitated by adding 40 μ g of glycogen, 30 μ L of 3 M sodium acetate, pH 5.2 and 900 μ L of 100% ethanol. Samples were incubated overnight at –20°C. The RNA was recovered by centrifugation, washed and suspended directly in 19.5 μ L of "Fragment, Prime, Finish (FPF)" mix from the TruSeq® Stranded mRNA LT kit to proceed to prepare libraries for deep sequencing according to the manufacturer's protocol (Illumina). HiSeq 50 Cycle Single-read sequencing was performed by the High Throughput Genomics Core Facility at the Huntsman Cancer Institute (University of Utah) on an Illumina HiSeq 2500 instrument.

Bioinformatic analyzes—A reference sequence was created using marmoset genomic sequence (calJac3 assembly), Saimiriine herpesvirus 2 (Genbank: X64346.1) and splice junction sequences. The splice junction sequences were generated using USeq (v8.8.8) MakeTranscriptome using default settings. CalJac3 Ensembl build 80 and X64346.1 annotations were used in MakeTranscriptome. Reads were aligned to the reference sequence using Novoalign (2.08.03) allowing up to 50 alignments and removing adapter sequences. The resulting alignments were then run through USeq SamTranscriptomeParser using default settings to convert splice alignments back to genomic space and to remove low quality reads. Reads aligning to multiple locations in the genome were removed. USeq DefinedRegionDifferentialSeq was used to count the number of aligned reads to each gene. Only reads aligning to the opposite strand of the annotation were counted. Genes with fewer than 10 reads across all replicates were thrown out. The resulting read counts were then run in DESeq (v1.4.5) to determine differentially expressed genes between each condition using default settings. Genes that were significantly enriched (Benjamini Hochberg adjusted p -value < 0.05 , $\log_2$ fold change > 0) in the HSUR 2-positive pull down versus HSUR 2-negative pull down and in the HSUR 2-positive pull down versus HSUR 2-positive input were used in further analyses (Supplementary Table 1). Significant genes were analyzed by Ingenuity Pathway Analysis version 24718999 (IPA, Qiagen) using common name, and compared against the ingenuity knowledge base. All pathways that showed a Fisher's Exact Test p -values < 0.05 (not adjusted for multiple hypothesis testing) are shown in Supplementary Table 2, whereas significant pathways that are relevant for HVS infection are shown in Fig. 2c.

Statistical analyses

No statistical methods were used to predetermine sample size, nor were the experiments randomized or the investigators blinded to sample allocation during experiments and evaluation of experimental results. Biological replicates (n) indicated in figures and figure legends refers to the number of independent experiments performed. The number of independent experiments were chosen to allow for statistical significance. Statistical analysis was performed using Graphpad Prism 7. Data were analyzed for the presence of outliers using the ROUT method ($Q=10\%$). Statistical analysis of biological replicates shown in Figures 2d and Extended data Fig. 3 was performed with Student's t -tests corrected for multiple comparisons with the Holm-Sidak method ($\alpha=0.05$) after using the method of Bartlett (<https://home.ubalt.edu/ntsbarsh/business-stat/otherapplets/BartlettTest.htm>) to determine whether the variance of the different groups was significantly different. Two-sided p -values of biological replicates shown in Figures 2e, 3, and extended Figs. 4d, 5, 8 and 9 were obtained with Student's t -tests compared to the control samples set at 1.0. When outliers were present the samples were compared with Wilcoxon's Signed Rank test. Pairwise comparisons between experimental groups and controls shown in Figs. 4 and extended Fig. 10 were performed with unpaired Student's t -test with or without Welch's correction for unequal variances after using the method of Bartlett to determine whether the variance of the different groups was significantly different. When outliers were present (ROUT method, $Q=10\%$) the paired groups were compared using the non-parametric Mann-Whitney test. For all data presented, please see Supplementary Table 7 for specific test used, exact number of replicates, and exact p -values.

Data availability

Source data for all figures in this article are included in its Supplementary Information. The described RNA-seq data have been deposited in the Gene Expression Omnibus under accession number GSE79082. Further data that support the findings of this study are available from the corresponding author upon reasonable request.

Extended Data

Extended Data Figure 1. HSUR 2 binds host miRNA miR-142-3p and miR-16
a, Nucleotide sequence of HSUR 2. Predicted base-pairing between HSUR 2 and miR-142-3p, as well as experimentally confirmed interaction between HSUR 2 and miR-16⁷ are shown with miRNA seed sequences in yellow and HSUR 2 residues involved in interactions with miRNAs in blue. AU-rich element (ARE)-like sequences are shown in red boxes, whereas the Sm-binding site is shown in a black box. **b**, Western blot analysis of miR-142-3p validated targets GRLF1³⁷, Rac1³⁸, Rock2³⁸, and N-WASP³⁹ in marmoset T cells transformed by wild-type HVS (cj319-WT) or by a mutant version of HVS lacking HSURs 1 and 2 (cj319-Δ2a). α-Tubulin provides a loading control. Data shown are representative of three independent experiments.

Extended Data Figure 2. AMT-mediated crosslinking and purification of HSUR 2 for RICC-seq
a, Northern blot analyses of purification of HSUR 2 for RICC-seq. Cj319-WT marmoset T cells were transiently transfected with Control ASO, and UV-crosslinked in the presence of

AMT. Total RNA was prepared, polyA+ was selected, and HSUR 2 was captured using a specific biotinylated ASO. RNA was isolated from different fractions, crosslinks were reversed, and analyzed by Northern blot for HSUR 2, or U1 snRNA as a crosslinking and purification control. FT: Flow-through, α H2: biotinylated HSUR2 ASO. **b**, Northern blot analyses of HSUR 2 and HSUR 7 (loading control) on total RNA prepared from cj319-WT cells transiently transfected with either Control ASO or HSUR 2 ASO. RNA samples prepared from these cells were subjected to the RICC-seq protocol as described in **a**.

Extended Data Figure 3. HSUR 2 regulates the abundance of target mRNAs
qRT-PCR analyses of HSUR 2 target mRNAs in cj319- 2a, which do not express HSUR 1 and HSUR 2^{10,21}, after transfection with either Control or HSUR 2 ASO ($n=3$; t -test, two-sided). Dots represent mean values of independent experiments with error bars representing s.d. (see Supplementary Table 7 for full description of statistics).

Extended Data Figure 4. HSURs 3, 4, 6, and 7 are not required for HSUR 2-mediated mRNA repression

a, Plasmid constructs used for transient transfection of HSURs. GFP (green arrow) expression is driven by the CMV promoter (blue box). HSUR encoding regions are represented by red arrows. HVS miRNAs²⁹ are not depicted. **b**, Plasmid constructs used for transient expression of HSUR 2. HSUR 2 expression signals (distal sequence element, DSE; proximal sequence element, PSE) are represented by magenta boxes, whereas the 3'-end processing signal (3'-box) is represented by a purple box. **c**, Northern blot analysis of GSML cells transiently transfected with pBS-GFP (lane 1), pBS-GFP-H1-5 (lane 2), pBS-GFP-H3-7 (lane 3), and both pBS-GFP-H1-5 and pBS-GFP-H3-7 (lane 4). U2 snRNA provides a loading control. **d**, GSML cells were transiently transfected with the plasmid constructs described in **b**, FACS-sorted for GFP expression, and analyzed as described in Fig. 2e (two-sided *t*-test in all cases except for NGDN, HSURs3-7 vs. empty vector: Wilcoxon, two-sided) (****p*<0.0001, ****p*<0.001, ***p*<0.01, **p*<0.05; see Supplementary Table 7 for full description of statistics).

Extended Data Figure 5. HSUR 2 represses target mRNAs in the human B cell line BJAB

BJAB cells were transiently transfected with plasmids pBS-GFP (Empty vector, white bars), pBS-GFP-HSUR2 (HSUR 2), or pBS-GFP-ΔHSUR2 (ΔHSUR2), FACS-sorted for GFP expression, and analyzed as described in Fig. 2e (*t*-test, two-sided). Dots represent mean values of independent experiments with error bars representing s.d. (****p*<0.001, ***p*<0.01, **p*<0.05; see Supplementary Table 7 for full description of statistics).

Extended Data Figure 6. Construction of cj137-WT and cj137- HSUR2 cell lines

a, Schematic representation of construction of HVS-BAC-GFP-WT and HVS-BAC-GFP-

HSUR2 bacmids. HVS-BAC-GFP was originally constructed using the HVS-A11-S4 strain^{31,33} with a cassette containing BAC elements, GFP gene, and chloramphenicol and hygromycin resistance genes placed within the ORF 15 gene³⁰. PCR fragments containing the kanamycin resistance gene and I-SceI restriction site flanked by a 50bp duplication of HVS genomic sequence (brown boxes) used for first recombination are depicted, with numbers between parentheses above ends indicating the corresponding positions in the HVS-A11 genome. After a second recombination event, scarless removal of the kanamycin cassette is achieved, leaving original 50 bp of genomic sequence (brown box). **b**, Restriction fragment patterns of recombinant BAC clones. Bacmids HVS-BAC-GFP, HVS-BAC-GFP-WT, and HVS-BAC-GFP- HSUR2 were digested with SacI, separated in a 0.7% agarose gel, and stained with ethidium bromide. Arrows indicate alterations due to addition of recombined fragments. Sizes of molecular weight markers (kb) are shown on the left. **c**, Northern blot analysis for HSUR expression on total RNA isolated from cj137-WT and cj137- HSUR2 cell lines. U6 snRNA provides a loading control.

Extended Data Figure 7. Ablation of miR-142-3p and miR-16 expression does not affect HSUR 2 expression nor its ability to base-pair with mRNAs

a, Northern blot analysis of total RNA isolated from GSML cells treated as described in Fig. 3a. U6 snRNA provides a loading control. **b**, AMT-mediated RNA-RNA crosslinking and polyA+ selection was performed as in Fig. 1a on HVS-transformed marmoset T cells transiently transfected with the LNA inhibitors indicated.

Extended Data Figure 8. Interaction of HSUR 2 with miR-142-3p and miR-16 is required for HSUR 2-mediated mRNA repression

a, Northern blot analysis of total RNA isolated from GSML cells that were treated as described in Fig. 3c. U6 snRNA provides a loading control. **b**, Same as in Fig. 3c for other targets of HSUR 2. Analyzed by two-sided *t*-test in all cases except for STAG1, RBM7, RASA2, DDX20, and DDX5 (HSUR2 Δ142-3p/16 vs. empty vector in all cases), for which a Wilcoxon Signed Rank test (two-sided) was used. Dots represent mean values of independent experiments with error bars representing s.d. (*****p*<0.0001, ****p*<0.001, ***p*<0.01, **p*<0.05; see Supplementary Table 7 for full description of statistics).

Extended Data Figure 9. Interaction of HSUR 2 with miR-142-3p and miR-16 is required for HSUR 2-mediated mRNA repression
a–b, Same as in Figs. 3d and 3e for other targets of HSUR 2 ($n=3$; t -test, two-sided). Dots represent mean values of independent experiments with error bars representing s.d. (** $p<0.001$, ** $p<0.01$, * $p<0.05$; see Supplementary Table 7 for full description of statistics).

Extended Data Figure 10. HSUR 2 confers resistance to apoptosis
a–b, Same as in Figs. 4c and 4d, but cells were treated with TNF- α ($n=3$; unpaired t -test, two-sided). Dots represent mean values of independent experiments with error bars representing s.d. (* $p<0.05$; see Supplementary Table 7 for full description of statistics).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. HSUR 2 binds to translationally active mRNAs

a, Northern blot analyzes of polyA+ RNA from HVS-transformed marmoset T cells that were *in vivo* UV crosslinked in the absence (lanes 2 and 3) or presence (lanes 4 and 5) of psoralen (AMT). I: Input (2%); S: Supernatant (2%); P: Pellet (100%). **b–d**, Northern blot analyzes of different RNAs in cytoplasmic extracts from HVS-transformed marmoset T cells left untreated (**b** and **c**), or incubated with either puromycin or pactamycin (**d**). The extract in **c** was treated for 20 min on ice with 50 mM EDTA. I: Input (20%). In each case, data shown are representative of three independent experiments.

Figure 2. RICC-seq identifies mRNAs that are targets of HSUR 2

a, Diagram of the strategy used for purification of RNAs that base-pair with HSUR 2 *in vivo*. **b**, Categories of transcripts identified by RICC-seq. **c**, Ingenuity pathway analysis showing selected pathways for HSUR 2 targets. Dashed red line demarcates significance threshold ($p < 0.05$). **d**, HSUR 2 target mRNA levels in HVS-transformed marmoset T cells (cj319-WT) after transfection with either Control or HSUR 2 ASO (*t*-test, two-sided). **e**, Same as in **d** in GSML cells FACS-sorted for green fluorescent protein (GFP) after transient transfection with empty vector carrying GFP or with a plasmid carrying GFP and either HSUR 2 driven by HSUR 2 promoter (HSUR2) or HSUR 2 promoter alone (Δ HSUR2, Extended data Fig. 4b) ($n=3$; *t*-test, two-sided). **f**, Western blot of HSUR 2 targets in marmoset T cells transformed with wild-type HVS (cj137-WT) or mutant HVS lacking HSUR 2 (cj137- Δ HSUR2). In each case, data shown are representative of three independent experiments. For **d** and **e**, dots represent mean values of independent experiments with error bars representing s.d. (**** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$; see Supplementary Table 7 for full description of statistics).

Figure 3. Interaction of HSUR 2 with miR-142-3p and miR-16 is required for HSUR 2-mediated mRNA repression

a, Enrichment of HSUR 2 target mRNAs in Ago immunoprecipitates from extracts prepared from cj319-WT cells transfected with either Control or HSUR 2 ASO ($n=5$ and two-sided t -test in all cases except for β -Actin: $n=4$, Wilcoxon). **b**, qRT-PCR analyses of HSUR 2 targets NGDN and RB1 in GSML cells transiently co-transfected with empty vector carrying GFP or with a plasmid carrying GFP plus HSUR 2, and with either a control LNA inhibitor or LNA inhibitors with complementarity to either miR-142-3p or miR-16 (t -test, two-sided). **c**, Same as in **b** after transient transfection with mutant versions of HSUR 2. Partial sequences of HSUR 2 (position indicated by number) with residues (in bold) involved in either base-pairing with miRNAs (shown in pink) or in ARE-like sequences, and mutations in such sequence, are shown (t -test, two-sided). **d-e**, Same as in **c**, but transiently co-transfected with Control miRNA or a mutant version of either miR-142-3p (miR-142-3p_{mut}; modified residues in blue) or miR-16 (miR-16_{mut}; modified residues in blue) that have complementarity to HSUR2 142-3p or HSUR2 16, respectively ($n=3$; t -test, two-sided). For **a-d**, dots represent mean values of independent experiments with error bars representing s.d. (** $p<0.001$, ** $p<0.01$, * $p<0.05$; see Supplementary Table 7 for full description of statistics).

Figure 4. HSUR 2 confers resistance to apoptosis

a, Flow cytometry analysis of *cj137*-WT and *cj137*- Δ HSUR2 cells stained with Annexin V-Alexa 647 and PI. Right panel shows quantitation of five independent experiments (Viable and Debris: unpaired *t*-test, two-sided; Necrotic and Apoptotic: Mann-Whitney, two-sided). **b**, Caspase activity measured in *cj137*-WT and *cj137*- Δ HSUR2 cells with the Caspase-Glo assay system ($n=3$; unpaired *t*-test, two-sided). **c**, Caspase-3/7 activity measured as in **b** in BJAB cells FACS-sorted after transient transfection with empty vector, or vectors expressing either HSUR 2 or mutant versions of HSUR 2 that cannot bind miR-142-3p and/or miR-16 (described in Fig. 3c) and incubated in the presence of soluble FAS ligand ($n=3$; unpaired *t*-test, two-sided). **d**, Same as in **c**, but transiently co-transfected either with Control miRNA, miR-142-3p_{mut}, or miR-16_{mut} ($n=3$; unpaired *t*-test, two-sided). **e**, Model of HSUR 2 function. HSUR 2 base-pairs with both target mRNAs and miR-142-3p and miR-16, resulting in tethering of these miRNAs to target mRNAs. HSUR-2 mediated recruitment of miR-142-3p and miR-16 results in repression of the expression of target mRNAs. For **a**–**d**, dots represent mean values of independent experiments with error bars representing s.d. (**** $p<0.0001$, * $p<0.05$; see Supplementary Table 7 for full description of statistics).