SUPPLEMENTARY FIGURES AND TABLES

S. Rüegger et al. The Ribonucleotidyl Transferase USIP-1 Acts with SART3 to Promote U6 snRNA Recycling. Nucleic Acids Res (2015)

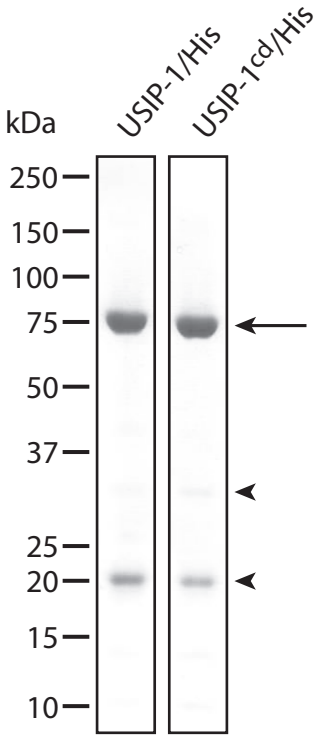

Figure S1. Purity of recombinant USIP-1.
Coomassie staining of recombinant USIP-1 and USIP-1cd purified through a C-terminal His-Tag using a HisTrap column. An arrow points to USIP-1, which has a calculated mass of 75 kDa; arrowheads point to residual contaminations.

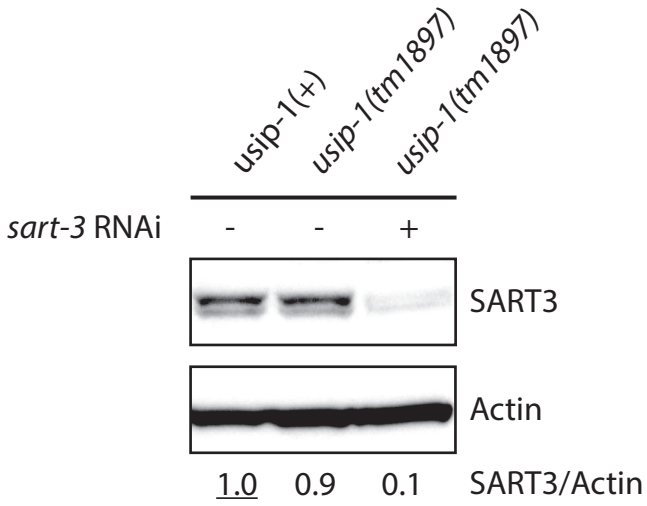

Figure S2. Knock-down efficiency of *sart-3*.
Western blot of lysates obtained from wild-type worms (*usip-1(+)*) exposed to mock RNAi (lane 1), *usip-1(tm1897)* worms exposed to mock RNAi (lane 2), and *usip-1(tm1897)* worms exposed to *sart-3* RNAi (lane 3). Quantification of band signals is relative to the underlined value, which has been set to 1.

Figure S3

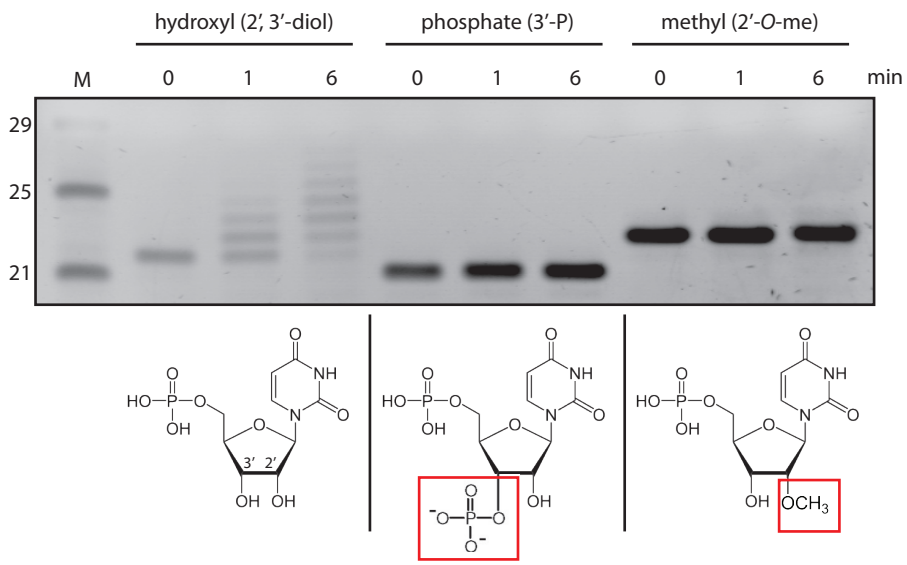

Figure S3. Activity of USIP-1 on different RNA 3' ends.
Transferase assay (see methods) adding recombinant USIP-1 to a synthetic 22-nucleotide-long RNA substrate containing a uridine at the 3' end that is either unmodified (2',3'-diol), 3'-monophosphorylated (3'-P), or 2'-*O*-methylated (2'-*O*-me). Note that the 2'-*O*-methylated substrate is 23 (not 22) nucleotides long containing an additional uridine at the 3' end. Shown is a SYBR Gold staining of a 15% urea-polyacrylamide gel (inverted picture). M = Marker

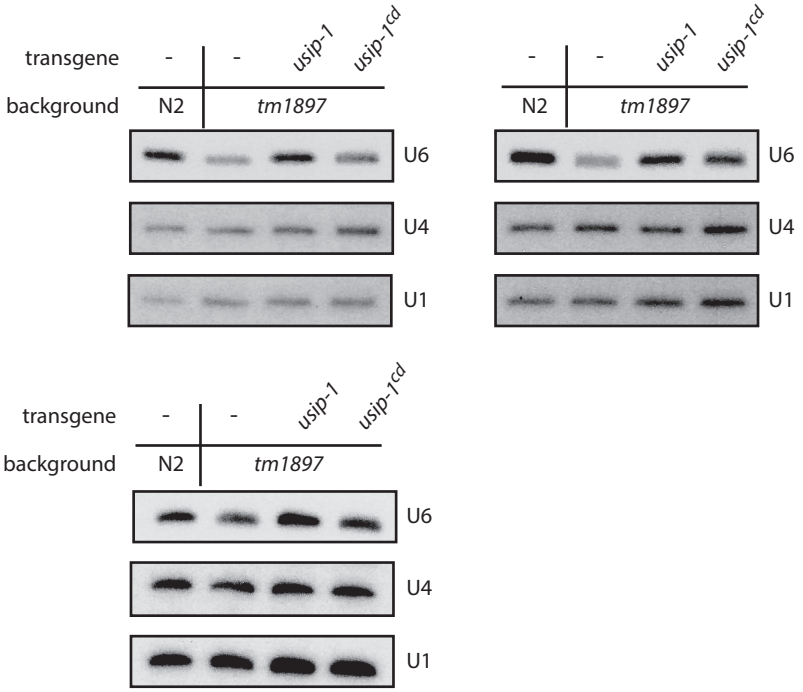

Figure S4. Replicate 2-4 of Northern blot shown in figure 6A.

Table S1. Coverage in percent of protein sequence of indicated proteins in IP1 and IP2 shown in figure 2B.

IP1

	FLAG/SART3		GFP/His/FLAG	
RNase A	no	yes	no	yes
SART3	57	50	0	0
GFP	0	0	58	46
GUT-2	80	0	0	0
LSM-3	75	0	0	0
LSM-4	28	0	0	0
LSM-5	87	0	0	0
LSM-6	36	0	0	0
LSM-7	33	0	0	0
LSM-8	84	0	0	0
SNR-1	50	0	0	0
SNR-2	33	0	0	0
SNR-3	25	0	0	0
SNR-4	59	0	0	0
SNR-5	42	0	0	0
SNR-7	61	0	0	0
USIP-1	19	0	0	0

IP2

	FLAG/SART3		GFP/His/FLAG	
RNase A	no	yes	no	yes
SART3	53	53	0	0
GFP	0	0	78	75
GUT-2	60	0	0	0
LSM-3	71	0	0	0
LSM-4	41	0	0	0
LSM-5	21	0	0	0
LSM-6	60	0	0	0
LSM-7	19	0	0	0
LSM-8	72	0	0	0
SNR-1	51	0	0	0
SNR-2	0	0	0	0
SNR-3	42	0	0	0
SNR-4	56	0	0	26
SNR-5	48	0	0	0
SNR-7	53	40	0	0
USIP-1	33	0	0	0

	IP1		IP2	
	LSM	SNR	LSM	SNR
average % coverage	60.4	45	49.1	41.7

Table S2. Additional proteins bound to USIP-1 and SART3.

Additional proteins identified by mass spectrometry in the co-IP shown in figure 3B enriched by ≥ 10-fold in either the USIP-1 or SART3 IP over the GFP/His/FLAG negative control. Table is ranked according to the USIP-1/GFP ratio. The numbers in the table indicate the quantity of spectra mapping uniquely to a given protein.

C. elegans	*H. sapiens*	*S. cerevisiae*	GFP/His/FLAG	USIP-1/GFP/3xFLAG	FLAG/SART3
swsn-1	SMARCC1	SWI3	0	28	8
atp-2	ATP5B	ATP2	0	25	8
pde-6	PDE8A	PDE2	0	24	6
hsp-6	HSPA9	SSC1	0	21	9
rpl-34	RPL34	RPL34B	0	18	11
rpn-2	PSMD1	RPN2	0	18	5
rpl-9	RPL9	RPL9B	0	17	11
rpn-3	PSMD3	RPN3	0	16	9
sip-1	CRYAB		0	16	0
rpl-26	RPL26	RPL26B	0	14	11
rpl-19	RPL19	RPL19A	0	14	5
rpl-32	RPL32	RPL32	0	13	9
atp-4	ATP5J		0	12	8
cct-5	CCT5	CCT5	0	12	5
copb-2	COPB2	SEC27	0	12	0
eef-2	EEF2	EFT1	0	11	6
nap-1	NAP1L1	NAP1	0	11	5
dlst-1	DLST	KGD2	0	11	5
rpn-8	PSMD7	RPN8	0	11	5
F18C12.3	SRFBP1	USO1	0	11	4
cpsf-1	CPSF1	CFT1	0	11	2
copa-1	COPA	COP1	0	11	0
rpl-28	RPL28		0	10	8
pas-4	PSMA7	PRE6	0	10	5
cgh-1	DDX6	DHH1	0	10	3
cey-1	YBX3	NOP1	0	6	10

Table S3. Worm strains

strain name	genotype	comment
gfp control		
HW781	EG5003, xeSi17[Pxrn-2::gfp::his::flag::xrn-2 3', unc-119(+)] IV	
sart-3 lines*		
HW1008	EG6701, xeSi55[Pdpy-30::sart-3::gfp::his::flag::xrn-2 3', unc-119(+)] I	
HW1337	sart-3(xe3)/nT1[qIs51] IV	
HW1338	HW1008; sart-3(xe3) IV	
HW1339	EG6699, xeSi126[Pdpy-30::flag::sart-3::gpd-operon::gfp::his-58::tbb-2 3', unc-119(+)] II	
HW1340	EG6699, xeEx386(WRM0622D_C09::gfp::3xflag; Pmyo-2::mCherry)	gfp::3xflag tagged B0035.12 (sart-3) on fosmid
HW1341	HW1339; sart-3(xe3)/nT1[qIs51] IV	
HW1350	unc-119(ed3) III; ttTi5820 IV	
usip-1 lines*		
HW1251	ZK863.4(tm1897) V	
HW1342	EG6699, xeEx387(WRM0610A_C05::gfp::3xflag)	gfp::3xflag tagged ZK863.4 (usip-1) on fosmid
HW1343	EG6699, xeSi127[Pdpy-30::ZK863.4::flag::gpd-operon::gfp::his-58::tbb-2 3'), unc-119(+)] II	
HW1344	EG6699, xeSi128[Pdpy-30::ZK863.4(D183A/D185A)::flag::gpd-operon::gfp::his-58::tbb-2 3'), unc-119(+)] II	
HW1345	HW1343; ZK863.4(tm1897) V	
HW1346	HW1344; ZK863.4(tm1897) V	

Table S4. Oligonucleotide primer & northern probe sequences

Lower case letters = gateway recombination sites

Cloning	**Forward (5'->3')**	**Reverse (5'->3')**
sart-3 (genomic DNA)	ggggacaagtttgtacaaaaaagcaggcttgATGTCCGATGTGGATATGG	ggggaccactttgtacaagaaagctgggtgATTTTTCATAAACATTTTACGG
flag::sart-3 (genomic DNA)	ggggacaagtttgtacaaaaaagcaggctgcATGGATTATAAAGATGATGATGACAAGTCCGATGTGGATATG	ggggaccactttgtacaagaaagctgggtgTTAATTTTTCATAAACATTTTAC
usip-1 (genomic DNA)	ggggacaagtttgtacaaaaaagcaggcttgATGTCTTCAAACTTGCAACTGG	ggggaccactttgtacaagaaagctgggtgTTATGGCCAAGTTGGGGCTGC
Genotyping		
tm1897	CGCCTCCGTGCGCACTTGAG	GTTATGCTGTGAAAACAAGC
Site-directed mutagenesis		
usip-1(D183A) (on genomic DNA)	GGCGAAATGGTTACTCAGCGATTGTAAGTTGTTAG	CTAACAACTTACAATCGCTGAGTAACCATTTCGCC
usip-1(D185A) (on genomic DNA)	CAATAATGTTTTTAGGCGATTAACGTGGAATCAG	CTGATTCCACGTTAATCGCCTAAAAACATTATTG
usip-1 amplicon 1 (on cDNA, Gibson cloning)	GGCTTCTGGCGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACC	CTGATTCCACGTTAATCGCAATCGCTGAGTAACCATTTCG
usip-1 amplicon 2 (on cDNA, Gibson cloning)	CGAAATGGTTACTCAGCGATTGCGATTAACGTGGAATCAG	CGAAATATTATGAGTAATATCCATGGGATCCATCAACATCAACGGAG
MosDEL		
left homology region	ggggacaactttgtatagaaaagttggcCGAACCATCTGAGTACGTCG	ggggactgctttttttgtacaaacttgcGCTCAAAAATGTGTTGCTTCTGG
right homology region	ggggacagctttcttgtacaaagtggcgCTTGCAGAATTTTGATGGAAAC	ggggacaactttgtataataaagttgcGAAGCTTTCCTACAAAGAGC
xe3 5' end insertion	CGTCCTCACTTTCTGAGCTG	CCAATTCATCCCGGTTTCTG
xe3 3' end insertion	CCAATTACTCTTCAACATCC	CGACATTATTGATGTAACACC
Probes for Nothern blotting		
U1	GCACGCAGCCCCGATACGCA	
U2	CGATAAGAACAGATACTACAC	
U4	CGCACCTCGGCAAAGCCTCA	
U5	GGTTAAATGCAGAGGAACCAGAGT	

U6	ATTTGCGTGTCATCCTTGCGCAGG	
tRNA(gly)	GCTTGGAAGGCATCCATGCTGACCATT	
3' RACE		
RT primer	GACCGAGTGTAGCAAGCGAGGACTCGAGCTCAAGCCAAGCAGAAGACGGCATACGA	
U6 amplification	GACCGAGTGTAGCAAGCG	GTTCTTCCGAGAACATATAC