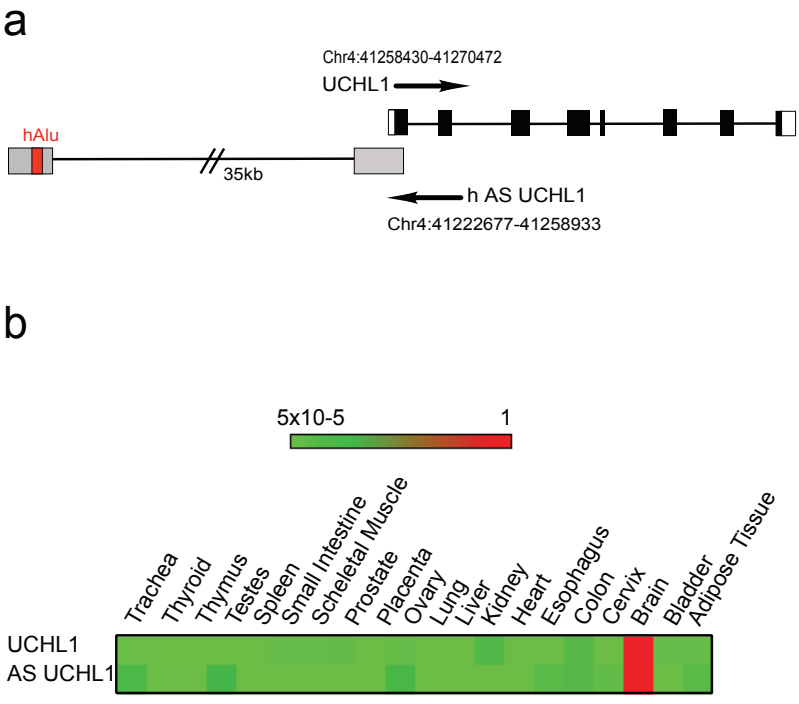
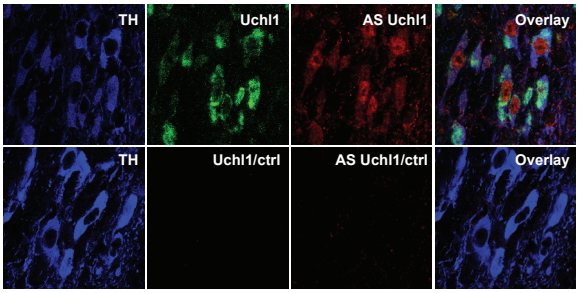
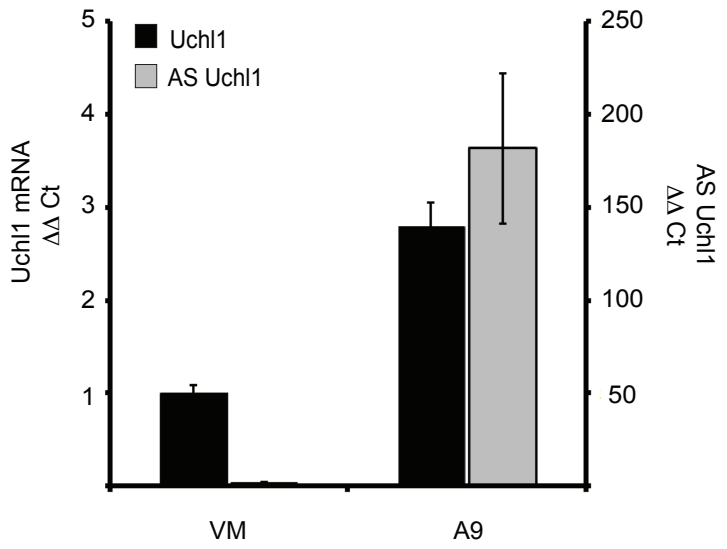
doi:10.1038/nature11508



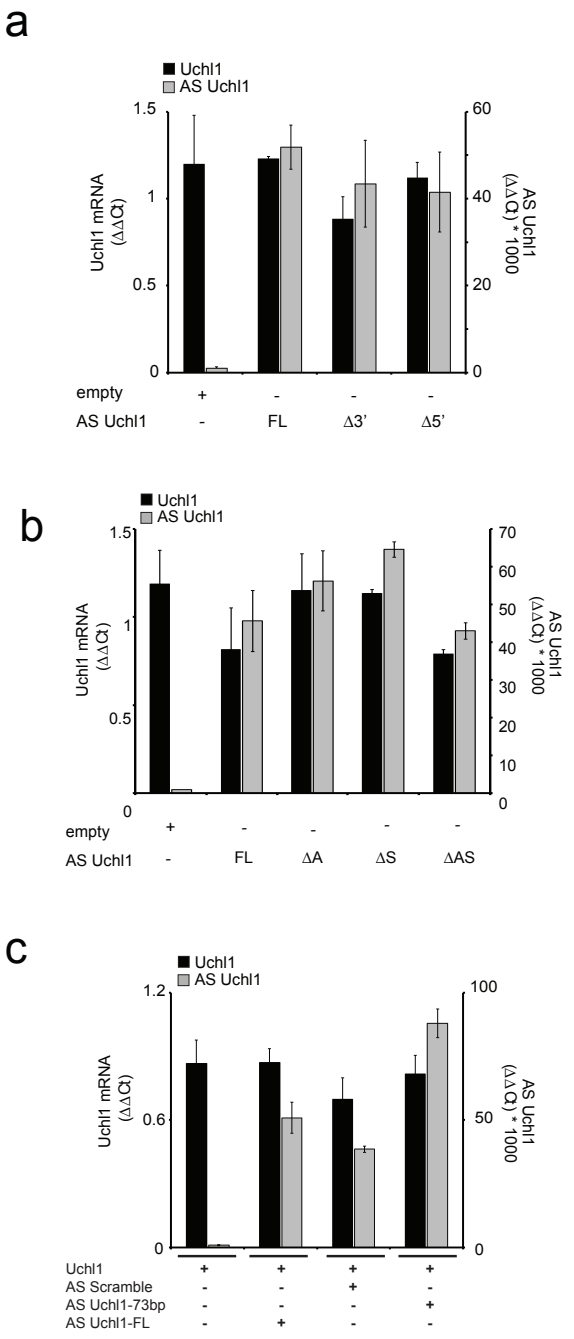
Supplementary Figure 1. Expression of antisense and sense UCHL1 in human tissues. a, Schematic diagram of human UCHL1/antisense (AS) UCHL1 genomic organization. UCHL1 exons are in black; 5' and 3' UTRs in white. AS UCHL1 exons are in grey; repetitive element (hAlu) in red. Introns are indicated as lines. **b,** Quantitative expression of UCHL1 and AS UCHL1 in human tissues. Heat map graphical representation of rescaled normalized fold expression (ΔΔCt/ΔΔCt MAX).



Supplementary Figure 2. Specificity of antisense Uchl1 expression in DA neurons of Substantia Nigra. Specificity of expression of Uchl1 sense (green) and antisense (AS) (red) transcripts (upper panels) as compared to control probes (Uchl1/ctrl and AS Uchl1/ctrl) (lower panels). DA neurons in Substantia Nigra are visualized by TH-staining in both panels (blue).



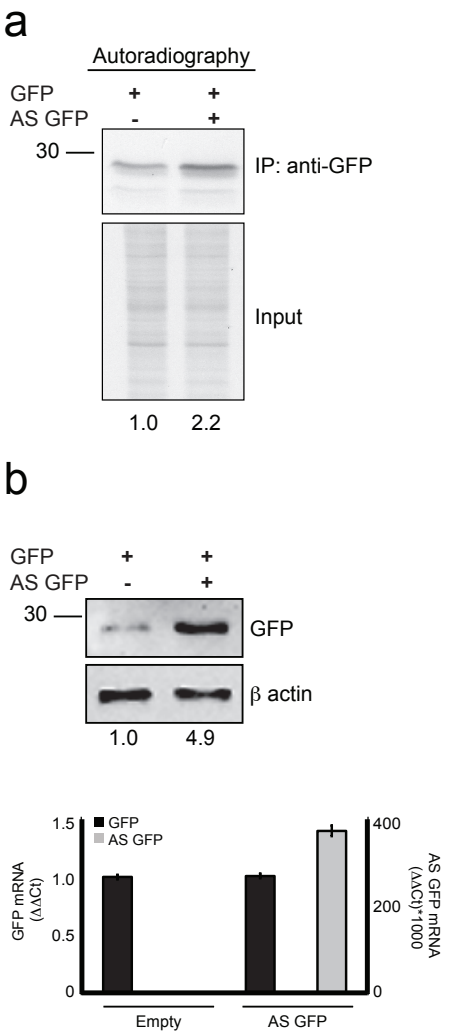
Supplementary Figure 3. Antisense Uchl1 is enriched in DA neurons. qRT-PCR starting from 300 LCM-isolated DA neurons from the Substantia Nigra of TH-GFP mice. Uchl1 and antisense (AS) Uchl1 were amplified with intron-spanning primers on three biological replica. ** $p<0.01$.



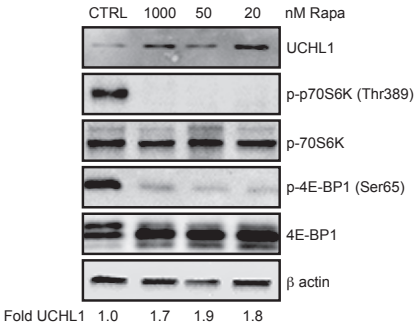
Supplementary Figure 4. Endogenous Uchl1 mRNA levels are not altered by overexpression of antisense Uchl1 mutants. **a,** qRT-PCR from MN9D cells transfected with Δ5' and Δ3' antisense (AS) Uchl1 mutants. Relative abundance of endogenous Uchl1 mRNA (left) and over-expressed deletion mutants (right) is shown. **b,** as in a) for ΔAlu (ΔA), ΔSINEB2(ΔS) and ΔAS mutants. **c,** as in a) for AS Uchl1-73bp. AS scramble and AS Uchl1-Full Length (FL) were used as negative and positive controls, respectively.

Riken Acc.	*AS to gene*	*NCBI Acc.*	*Orientation*	*Type*
AK019925	Ccdc44	NM_027346	RC	SINE/B2 #B3
AK029359	Uxt	NM_013840	RC	SINE/B2 #B3
AK032194	Nars2	NM_153591	RC	SINE/B2 #B3
AK032215	Nudt9	NM_028794	RC	SINE/B2 #B3
AK034331	n/a	NM_001012311	RC	SINE/B2 #B3
AK035015	Nrm	NM_134122	RC	SINE/B2 #B3
AK035406	Sv2b	NM_153579	RC	SINE/B2 #B3
AK041236	Ccdc88a	NM_176841	RC	SINE/B2 #B3
AK041654	Rcc	NM_133878	RC	SINE/B2 #B3
AK041742	Abhd11	NM_145215	RC	SINE/B2 #B3
AK042861	Wfdc5	NM_145369	RC	SINE/B2 #B3
AK044205	Rhod	NM_007485	RC	SINE/B2 #B3
AK045677	Eln	NM_007925	RC	SINE/B2 #B3
AK046828	n/a	NM_177006	RC	SINE/B2 #B3
AK047213	Uhmk1	NM_010633	RC	SINE/B2 #B3
AK048309	Epb4.9	NM_013514	RC	SINE/B2 #B3
AK053130	Rabgap1l	NM_001038621	RC	SINE/B2 #B3
AK054076	Gadd45a	NM_007836	RC	SINE/B2 #B3
AK078161	Nck1	NM_010878	RC	SINE/B2 #B3
AK078321	Uchl1	NM_011670	RC	SINE/B2 #B3
AK080749	Pgls	NM_025396	RC	SINE/B2 #B3
AK090347	3110005G23Rik	NM_028427	RC	SINE/B2 #B3
AK132441	A130022J15Rik	NM_175313	RC	SINE/B2 #B3
AK135599	Ednra	NM_010332	RC	SINE/B2 #B3
AK143014	Cdkn2aip	NM_172407	RC	SINE/B2 #B3
AK143784	Txnip	NM_001009935	RC	SINE/B2 #B3
AK145079	Gsk3b	NM_019827	RC	SINE/B2 #B3
AK149843	Cmtm6	NM_026036	RC	SINE/B2 #B3
AK163105	E4f1	NM_007893	RC	SINE/B2 #B3
AK165234	Dtx3	NM_030714	RC	SINE/B2 #B3
AK169421	n/a	NM_001110101	RC	SINE/B2 #B3

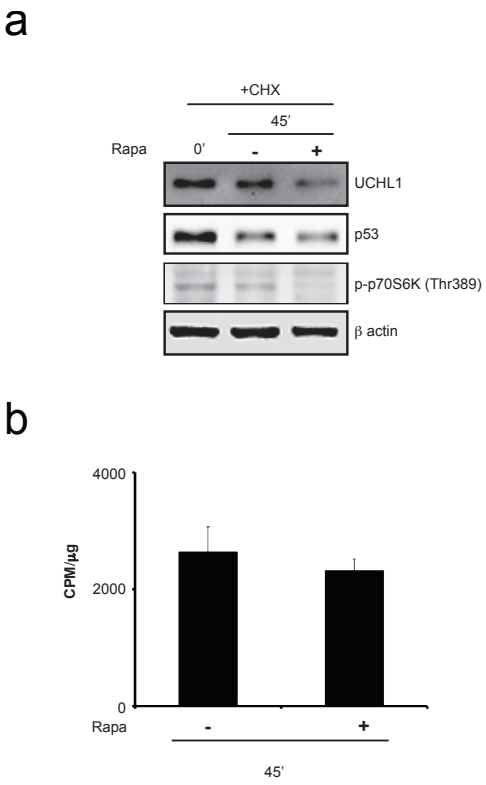
Supplementary Figure 5. Family of transcripts with embedded SINEB2. Family of FANTOM3 non-coding RNA clones that are antisense to protein coding genes and contain embedded SINEB2 in inverted orientation. In red, S/AS pairs tested in the manuscript.



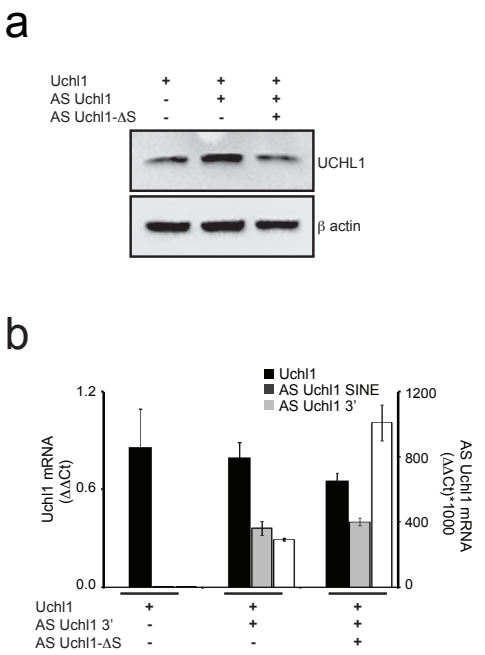
Supplementary Figure 6. Antisense GFP enhances GFP *de novo* **synthesis.** HEK cells were transfected with pEGFP plasmid and control vector (-) or antisense GFP (AS GFP), as indicated. Twenty-four hours after transfection cells were pulse-labeled with [35S]-Methionine/Cysteine for one hour. Labeled cells were harvested, lysed and immunoprecipitated with anti-GFP antibody. An aliquote of protein extract was used to monitor inputs. **a,** Autoradiography of IP and input. Bands of translated GFP were quantified relative to inputs. **b,** Expression of transfected constructs was analyzed in the same cells by western blotting to detect GFP protein (upper panel), and qRT-PCR to detect GFP and AS GFP transcripts (lower graph). For western analysis, actin served as a loading control. For qPCR, expression was normalized to actin, and mRNA levels in cells transfected with GFP plus empty vector was set to 1.



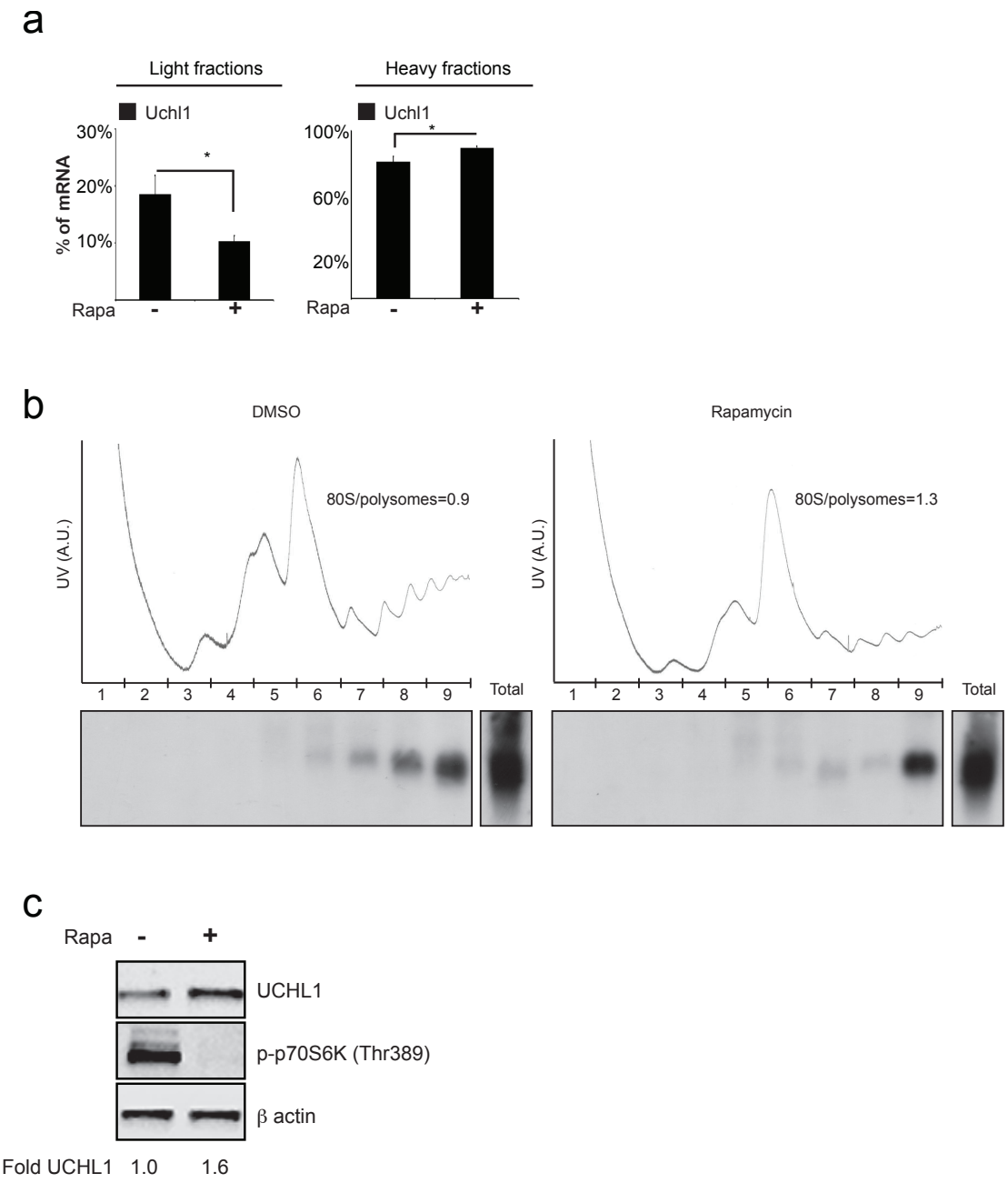
Supplementary Figure 7. Titration of rapamycin treatment on UCHL1 up-regulation. MN9D cells were treated with decreasing concentration of rapamycin (as indicated) for 45 minutes. Cell lysates were analyzed by western blot. Inhibition of mTOR was verified with anti-p-p70S6K (Thr389) and anti-p-4E-BP1 (Ser65) antibodies. Quantification of UCHL1 fold-induction is indicated.



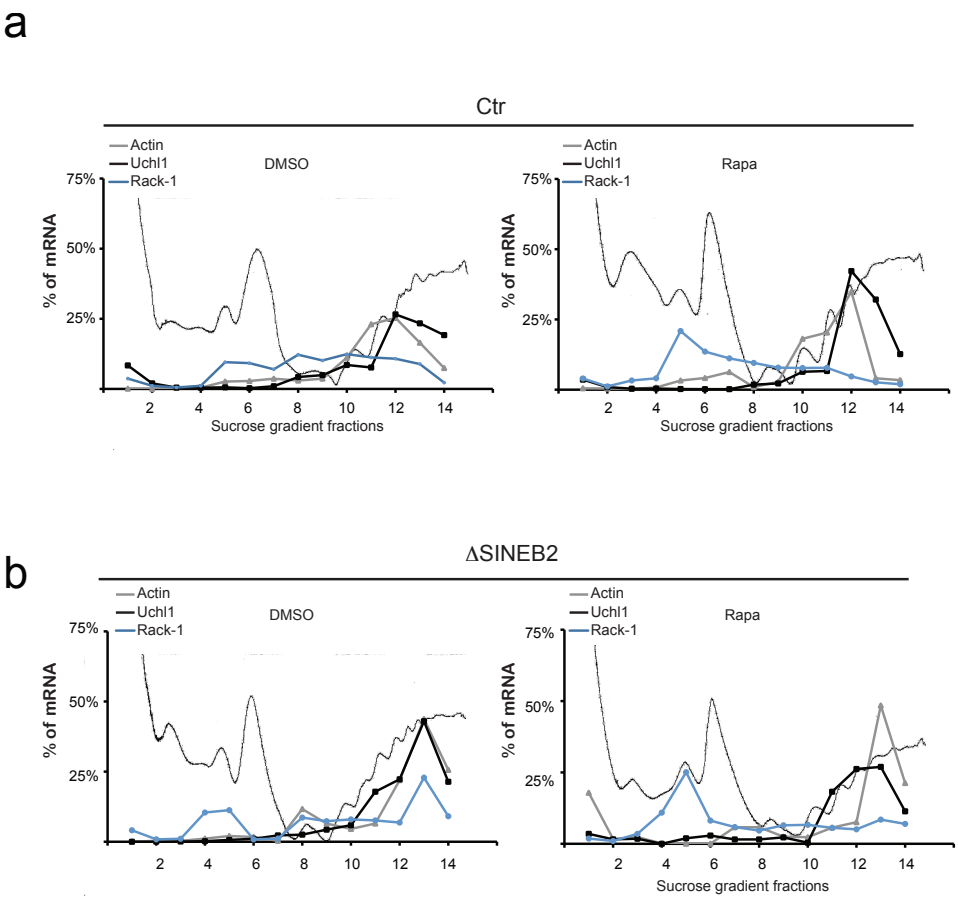
Supplementary Figure 8. Rapamycin treatment does not increase UCHL1 protein stabilization and global protein synthesis. a, UchL1 stability in MN9D cells treated with DMSO (CTRL) or with 1μM Rapamycin for 45 minutes, in the presence of 100μM Cyclohexamide (+CHX). p53 decay was used as positive control of CHX-dependent inhibition of translation. p-p70S6K (Thr389) was used as positive control for Rapamycin treatment. **b,** S35-Methyonine incorporation in MN9D cells treated with DMSO (-) or 1μM Rapamycin (+) for 45 minutes.



Supplementary Figure 9. Dominant-negative activity of antisense Uchl1-ΔSINEB2 (ΔS) over antisense Uchl1. a, Antisense (AS) Uchl1-ΔS acts as dominant-negative on AS Uchl1-mediated upregulation of UCHL1 protein levels in transfected HEK cells. **b,** No differences in Uchl1 mRNA levels upon AS Uchl1 or AS Uchl1-ΔS mutant expression.



Supplementary Figure 10. Uchl1 mRNA increases association to heavy polysomes upon rapamycin treatment. MN9D pcDNA 3.1 stable control cells were treated with rapamycin (Rapa) or vehicle alone (DMSO). **a**, Uchl1 mRNA was measured in sucrose gradient fractions by qRT-PCR. Quantification of Uchl1 mRNA signals is obtained by the addition of the fractions corresponding to the light subpolysomal mRNPs and the heavy polysomes from three replica. Data indicate mean ± s.d. *p<0.05. **b**, RNA purified from 9 sucrose gradient fractions was used for Northern Blot analysis with radioactive riboprobes for Uchl1. **c**, Total protein lysates from the same preparation were analysed for UCHL1, p-p70S6K (Thr389) and beta-actin.



Supplementary Figure 11. Uchl1 mRNA association to heavy polysomes depends on a functional antisense Uchl1. qRT-PCR for **a,** MN9D pcDNA 3.1 stable control cells (Ctr) and **b,** stable antisense Uchl1 ΔSINEB2 mutant (ΔSINEB2) treated with rapamycin or vehicle alone (DMSO) was performed on RNAs purified from 14 sucrose gradient fractions. Association with each fraction is shown as linear plot of the percentage of RNA present in each fraction; the plot is superimposed on the absorbance profile of the gradient. Rack-1 is a TOP mRNA whose translation is specifically inhibited by rapamycin.

Cloning Oligo Name	**Sequence (5'->3')**
For mAS Uchl1 fl	ACAAAGCTCAGCCCACACGT
Rev mAS Uchl1fl	CATAGGAGTGTTTCATT
Human 5'F	AGATAATCTGGTGGTTGTGGAGAC
Human 3'R	CAGATGACATGCCAAAGAGATTAC
AS Uchl1 ShRNA	CGCGCAGTGACACAGCACA
For mAS Uchl1 Δ5'	CAGTGCTAGAGGAGGTCAGAAGAG
Rev mAS Uchl1 Δ3'	TGGCTTGTACCATTCTGTGC
For mAS Uchl1 fl Nhe	TATAGCTAGCACAAAGCTCAGCCCACACGT
Rev pre-SINE B2 EcoRI	GAGAGAATTCCAATGGATTCCATGT
For post-ALU EcoRI	GAGAGAATTCGATATAAGGAGAATCTG
Rev mAS fl HindIII	GCGCAAGCTTCATAGGGTTCATT
Rev pre-ALU EcoRI	GAGAGAATTCTTATAGTATGTGTTGTC
For post-ALU	GATATAAGGAGAATCTG
Rev pre-SINE B2 EcoRI	GAGAGAATTCCAATGGATTCCATGT
For post-SINE B2 EcoRI	GAGAGAATTCCTCCAGTCTCTTA
For SINE B2 inside	GAGAGTGCAGTGCTAGAGGAGG
Rev Alu flip	GAGACTCCCAGTCAGGCAATCC
Rev SINE flip	TATAGGAGCTAAAGAGATGGC
AS Uchl1 73bp F	GAGACTCGAGCTCGGGGTTAATCTCCATCGGCTTCAGCTGCATCTTCGCGGATGG
AS Uchl1 73bp R	ATATGATATCCGGCTCCTCGGGTTTGTGTCTGCAGGTGCCATCCGCGAAGATG
AS SCR F	ATATCTCGAGACATCACCCCAAGAAAAGCGGGAACGGTAGCTGGGTCTTGTTAAGATT
AS SCR R	GAGAGATATCCCTCGTTCCGATGGTTAAGACTCGGAATCTTAACAAGACCCAG
For mAS Uxt	TAGTCTCGCTGCAGTACG
Rev mAS Uxt	CATTATCTTCT
GFP AS F	ATATCTCGAGCCCGGTGAACAGCTCCTCGCCCTTGCTCACCATGGTGGCGACCGGTAGC
GFP AS R	GAGAGATATCTAGTGAACCGTCAGATCCGCTAGCGCTACCGGTCGCCACCATGGTGA
qRT-PCR Oligo Name	**Sequence (5'->3')**
mGapdh F	GCAGTGGCAAAGTGGAGATT
mGapdh R	GCAGAAGGGGCGGAGATGAT
mβ-actin F	CACACCCGCCACCAGTTC
mβ-actin R	CCCATTCCCACCATCACACC
mTBP S	TCAGTTCTGGAAAAATGGTGTG
mTBP AS	TGCTGCTAGTCTGGATTGTTCT
mRPII S	AATCCGCATCATGAACAGTG
mRPII AS	TCATCCATTTTATCCACCACCT
mAS Uchl1 F (Primer 3' F)	CTGGTGTGTATTATCTCTTATGC
mAS Uchl1 R (Primer 3' R)	CTCCCGAGTCTCTGTAGC
mAS Uchl1 SINE F	GGATATTGAGTTCCAAACACTGGT
mAS Uchl1 SINE R	TTCTCCTTATATCTCCCAGTCAGG
mAS Uchl1 overlap F (Primer 5' F)	GCACCTGCAGACACAAACC
mAS Uchl1 overlap R (Primer 5' R)	TCTCTCAGCTGCTGGAATCA
mUchl1 F	CCCCGAAGATAGAGCCAAG
mUchl1 R	ATGGTTCACTGGAAAGGG
mAS Uchl1 pre RNA F	CCATGCACCGCACAGAATG
mAS Uchl1 pre RNA R	GAAAGCTCCCTCAAATAGGC
mPre_ribosomal RNA F	TGTGGTGTCCAAGTGTTCATGC
mPre ribosomal RNA R	CGGAGCACCACATCGATCTAAG
mAS_Uxt F	CAACGTTGGGGATGACTTCT
mAS Uxt R	TCGATTCCCATTACCCACAT
mUxt F	TTGAGCGACTCCAGGAAACT
mUxt R	GAGTCCTGGTGAGGCTGTC
GFP F	GCCCGACAACCACTACCTGAG
GFP R	CGGCGGTCACGAACTCCAG
hUCHL1 S	GCCAATAATCAAGACAAAC
hUCHL1 AS	CATTCGTCCATCAAGTTC
hAS UCHL1 S	AAACCCATCCTTTCACCATCC
hAS UCHL1 AS	TTCCTATCTTCAGCCACATCAC
hTBP S 5'	TTCGGAGAGTTCTGGGATTGTA
hTBP AS 5'	TGGACTGTTCTTCACTCTTGGC
hRPII S 5'	GCACCACGTCCAATGACAT
hRPII AS 5'	GTGCGGCTGCTTCCATAA

Supplementary Figure 12. List of primers. Complete list of oligonucleotides used for cloning and quantitative PCR experiments.