

SUPPLEMENTARY DATA

A translational silencing function of MCPIP1/Regnase-1 specified by the target site context.

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Table S1. List of species and associated identification numbers used in conservation analysis of the TSE.

species	full species name	gi number	accession number
<i>A. carolinensis</i>	<i>Anolis carolinensis</i>	637285089	XM_008107807.1
<i>A. chloris</i>	<i>Acanthisitta chloris</i>	678009660	XM_009082357.1
<i>A. forsteri</i>	<i>Aptenodytes forsteri</i>	686587166	XM_009289724.1
<i>A. mexicanus</i>	<i>Astyanax mexicanus</i>	597782694	XM_007254824.1
<i>A. mississippiensis</i>	<i>Alligator mississippiensis</i>	564266010	XM_006271770.1
<i>A. platyrhynchos</i>	<i>Anas platyrhynchos</i>	514705285	XM_005009701.1
<i>A. sinensis</i>	<i>Alligator sinensis</i>	557321715	XM_006033903.1
<i>A. vittatum</i>	<i>Apaloderma vittatum</i>	699577453	XM_009878532.1
<i>B. a. scammoni</i>	<i>Balaenoptera acutorostrata scammoni</i>	594618588	XM_007163993.1
<i>B. b. bison</i>	<i>Bison bison bison</i>	742133263	XM_010842003.1
<i>B. bubalis</i>	<i>Bubalus bubalis</i>	594052444	XM_006050474.1
<i>B. mutus</i>	<i>Bos mutus</i>	555955948	XM_005890067.1
<i>B. r. gibbericeps</i>	<i>Balearica regulorum gibbericeps</i>	723539256	XM_010311855.1
<i>B. r. silvestris</i>	<i>Buceros rhinoceros silvestris</i>	704171263	XM_010133582.1
<i>B. taurus</i>	<i>Bos taurus</i>	41386700	NM_174726.1
<i>C. asiatica</i>	<i>Chrysochloris asiatica</i>	586476268	XM_006868625.1
<i>C. brachyrhynchos</i>	<i>Corvus brachyrhynchos</i>	669275494	XM_008631716.1
<i>C. c. cornix</i>	<i>Corvus cornix cornix</i>	727008523	XM_010395873.1
<i>C. canorus</i>	<i>Cuculus canorus</i>	696965093	XM_009555242.1
<i>C. carolinensis</i>	<i>Caprimulgus carolinensis</i>	704289793	XM_010177481.1
<i>C. cristata</i>	<i>Cariama cristata</i>	698462791	XM_009705669.1
<i>C. cristata</i>	<i>Condylura cristata</i>	507928084	XM_004675240.1
<i>C. ferus</i>	<i>Camelus ferus</i>	560924814	XM_006188533.1
<i>C. griseus</i>	<i>Cricetulus griseus</i>	625208064	XM_007646940.1
<i>C. hircus</i>	<i>Capra hircus</i>	548449541	XM_005674872.1
<i>C. jacchus</i>	<i>Callithrix jacchus</i>	675732486	XM_002807620.2
<i>C. lanigera</i>	<i>Chinchilla lanigera</i>	533143673	XM_005386339.1
<i>C. livia</i>	<i>Columba livia</i>	543731385	XM_005506682.1
<i>C. macqueenii</i>	<i>Chlamydotis macqueenii</i>	705674864	XM_010118153.1
<i>C. millii</i>	<i>Callorhinchus millii</i>	632956469	XM_007895783.1
<i>C. mydas</i>	<i>Chelonia mydas</i>	591373951	XM_007061607.1
<i>C. p. bellii</i>	<i>Chrysemys picta bellii</i>	641750357	XM_005283577.2
<i>C. pelagica</i>	<i>Chaetura pelagica</i>	701370516	XM_009993397.1
<i>C. porcellus</i>	<i>Cavia porcellus</i>	514459794	XM_003469287.2
<i>C. s. simum</i>	<i>Ceratotherium simum simum</i>	478531746	XM_004439967.1
<i>C. striatus</i>	<i>Colius striatus</i>	706113353	XM_010195349.1
<i>C. vociferus</i>	<i>Charadrius vociferus</i>	699695233	XM_009891638.1
<i>D. novemcinctus</i>	<i>Dasypus novemcinctus</i>	488552088	XM_004467418.1
<i>E. europaeus</i>	<i>Erinaceus europaeus</i>	617645658	XM_007532614.1
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<i>E. helias</i>	<i>Eurypyga helias</i>	704233408	XM_010157448.1
<i>E. przewalskii</i>	<i>Equus przewalskii</i>	664750538	XM_008534482.1
<i>E. telfairi</i>	<i>Echinops telfairi</i>	507661827	XM_004706379.1
<i>E. garzetta</i>	<i>Egretta garzetta</i>	697840280	XM_009642204.1
<i>F. albicollis</i>	<i>Ficedula albicollis</i>	524981453	XM_005038697.1
<i>F. catus</i>	<i>Felis catus</i>	586999163	XM_003991592.2
<i>F. cherrug</i>	<i>Falco cherrug</i>	541974912	XM_005440549.1
<i>F. damarensis</i>	<i>Fukomys damarensis</i>	731199713	XM_010614605.1
<i>F. glacialis</i>	<i>Fulmarus glacialis</i>	697017533	XM_009588062.1
<i>F. peregrinus</i>	<i>Falco peregrinus</i>	529435544	XM_005237489.1
<i>G. fortis</i>	<i>Geospiza fortis</i>	543273501	XM_005425605.1
<i>G. g. gorilla</i>	<i>Gorilla gorilla gorilla</i>	426341454	XM_004036004.1
<i>G. gallus</i>	<i>Gallus gallus</i>	57525457	NM_001006254.1
<i>G. stellata</i>	<i>Gavia stellata</i>	698396533	XM_009809398.1
<i>G. variegatus</i>	<i>Galeopterus variegatus</i>	667253664	XM_008566698.1
<i>H. albicilla</i>	<i>Haliaeetus albicilla</i>	700342099	XM_009916570.1
<i>H. glaber</i>	<i>Heterocephalus glaber</i>	512918106	XM_004903641.1
<i>H. leucocephalus</i>	<i>Haliaeetus leucocephalus</i>	729738876	XM_010567326.1
<i>H. sapiens</i>	<i>Homo sapiens</i>	299890852	NM_001005474.2

species	full species name	gi number	accession number
<i>J. jaculus</i>	<i>Jaculus jaculus</i>	507573859	XM_004670456.1
<i>L. africana</i>	<i>Loxodonta africana</i>	731506398	XM_003418969.2
<i>L. Chalumnae</i>	<i>Latimeria chalumnae</i>	557011970	XM_006006506.1
<i>L. discolor</i>	<i>Leptosomus discolor</i>	700424275	XM_009952236.1
<i>L. vexillifer</i>	<i>Lipotes vexillifer</i>	602678585	XM_007445548.1
<i>M. auratus</i>	<i>Mesocricetus auratus</i>	524949664	XM_005074792.1
<i>M. brandtii</i>	<i>Myotis brandtii</i>	554546942	XM_005867965.1
<i>M. davidii</i>	<i>Myotis davidii</i>	584056176	XM_006772587.1
<i>M. domestica</i>	<i>Monodelphis domestica</i>	612017192	XM_001363505.3
<i>M. gallopavo</i>	<i>Meleagris gallopavo</i>	733872178	XM_010720468.1
<i>M. lucifugus</i>	<i>Myotis lucifugus</i>	558151889	XM_006110123.1
<i>M. mulatta</i>	<i>Macaca mulatta</i>	297284898	XM_001099425.2
<i>M. musculus</i>	<i>Mus musculus</i>	226874842	NM_001159395.1
<i>M. nubicus</i>	<i>Merops nubicus</i>	675625854	XM_008942980.1
<i>M. ochrogaster</i>	<i>Microtus ochrogaster</i>	532001511	XM_005345064.1
<i>M. p. furo</i>	<i>Mustela putorius furo</i>	511905129	XM_004771986.1
<i>M. undulantes</i>	<i>Melopsittacus undulantes</i>	527266970	XM_005151575.1
<i>M. unicolor</i>	<i>Mesitornis unicolor</i>	704578053	XM_010189274.1
<i>M. vitellinus</i>	<i>Manacus vitellinus</i>	675405691	XM_008932261.1
<i>N. galili</i>	<i>Nannospalax galili</i>	674033563	XM_008835799.1
<i>N. leucogenys</i>	<i>Nomascus leucogenys</i>	441664675	XM_003261759.2
<i>N. nippon</i>	<i>Nipponia nippon</i>	694847778	XM_009466713.1
<i>N. notabilis</i>	<i>Nestor notabilis</i>	701300557	XM_010012428.1
<i>O. a. afer</i>	<i>Orycteropus afer afer</i>	634861974	XM_007945766.1
<i>O. anatinus</i>	<i>Ornithorhynchus anatinus</i>	620965882	XM_001514146.2
<i>O. aries</i>	<i>Ovis aries</i>	426217398	XM_004002892.1
<i>O. cuniculus</i>	<i>Oryctolagus cuniculus</i>	655855154	XM_008267009.1
<i>O. garnettii</i>	<i>Otolemur garnettii</i>	395858979	XM_003801777.1
<i>O. hoazin</i>	<i>Opisthocomus hoazin</i>	700394409	XM_009939434.1
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<i>O. princeps</i>	<i>Ochotona princeps</i>	504166967	XM_004593219.1
<i>O. r. divergens</i>	<i>Odobenus rosmarus divergens</i>	472343884	XM_004391639.1
<i>P. abelii</i>	<i>Pongo abelii</i>	686711529	XM_002813337.2
<i>P. adeliae</i>	<i>Pygoscelis adeliae</i>	690439637	XM_009322565.1
<i>P. alecto</i>	<i>Pteropus alecto</i>	586559710	XM_006913068.1
<i>P. anubis</i>	<i>Papio anubis</i>	685522586	XM_003893880.2
<i>P. bivittatus</i>	<i>Python bivittatus</i>	602645798	XM_007429274.1
<i>P. carbo</i>	<i>Phalacrocorax carbo</i>	695142022	XM_009515266.1
<i>P. crispus</i>	<i>Pelecanus crispus</i>	694631726	XM_009493355.1
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<i>P. humilis</i>	<i>Pseudopodoces humilis</i>	543360933	XM_005523961.1
<i>P. lepturus</i>	<i>Phaethon lepturus</i>	723123471	XM_010295033.1
<i>P. m. bairdii</i>	<i>Peromyscus maniculatus bairdii</i>	589968227	XM_006996546.1
<i>P. paniscus</i>	<i>Pan paniscus</i>	675766614	XM_008975426.1
<i>P. pubescens</i>	<i>Picoides pubescens</i>	699645934	XM_009896993.1
<i>P. sinensis</i>	<i>Pelodiscus sinensis</i>	558133331	XM_006116790.1
<i>P. t. altaica</i>	<i>Panthera tigris altaica</i>	591341175	XM_007095979.1
<i>P. troglodytes</i>	<i>Pan troglodytes</i>	694903186	XM_516633.5
<i>R. norvegicus</i>	<i>Rattus norvegicus</i>	157819394	NM_001107095.1
<i>R. roxellana</i>	<i>Rhinopithecus roxellana</i>	724951240	XM_010353332.1
<i>S. araneus</i>	<i>Sorex araneus</i>	505795446	XM_004606739.1
<i>S. b. boliviensis</i>	<i>Saimiri boliviensis boliviensis</i>	725595798	XM_010350049.1
<i>S. c. australis</i>	<i>Struthio camelus australis</i>	697483711	XM_009675099.1
<i>S. canaria</i>	<i>Serinus canaria</i>	683927126	XM_009095451.1
<i>S. harrisii</i>	<i>Sarcophilus harrisii</i>	395518880	XM_003763537.1
<i>T. alba</i>	<i>Tyto alba</i>	701348258	XM_009976113.1
<i>T. chinensis</i>	<i>Tupaia chinensis</i>	562828100	XM_006143585.1
<i>T. erythrolophus</i>	<i>Tauraco erythrolophus</i>	701314899	XM_009978950.1

species	full species name	gi number	accession number
<i>T. guttata</i>	<i>Taeniopygia guttata</i>	449486030	XM_004186221.1
<i>T. guttatus</i>	<i>Tinamus guttatus</i>	719729863	XM_010216978.1
<i>T. m. latirostris</i>	<i>Trichechus manatus latirostris</i>	471380818	XM_004378148.1
<i>T. syrichta</i>	<i>Tarsius syrichta</i>	640833928	XM_008074461.1
<i>U. maritimus</i>	<i>Ursus maritimus</i>	671018864	XM_008702036.1
<i>V. pacos</i>	<i>Vicugna pacos</i>	560971477	XM_006208029.1
<i>X. S. tropicalis</i>	<i>Xenopus (Silurana) tropicalis</i>	194332466	NM_001130266.1
<i>Z. albicollis</i>	<i>Zonotrichia albicollis</i>	542163629	XM_005490399.1

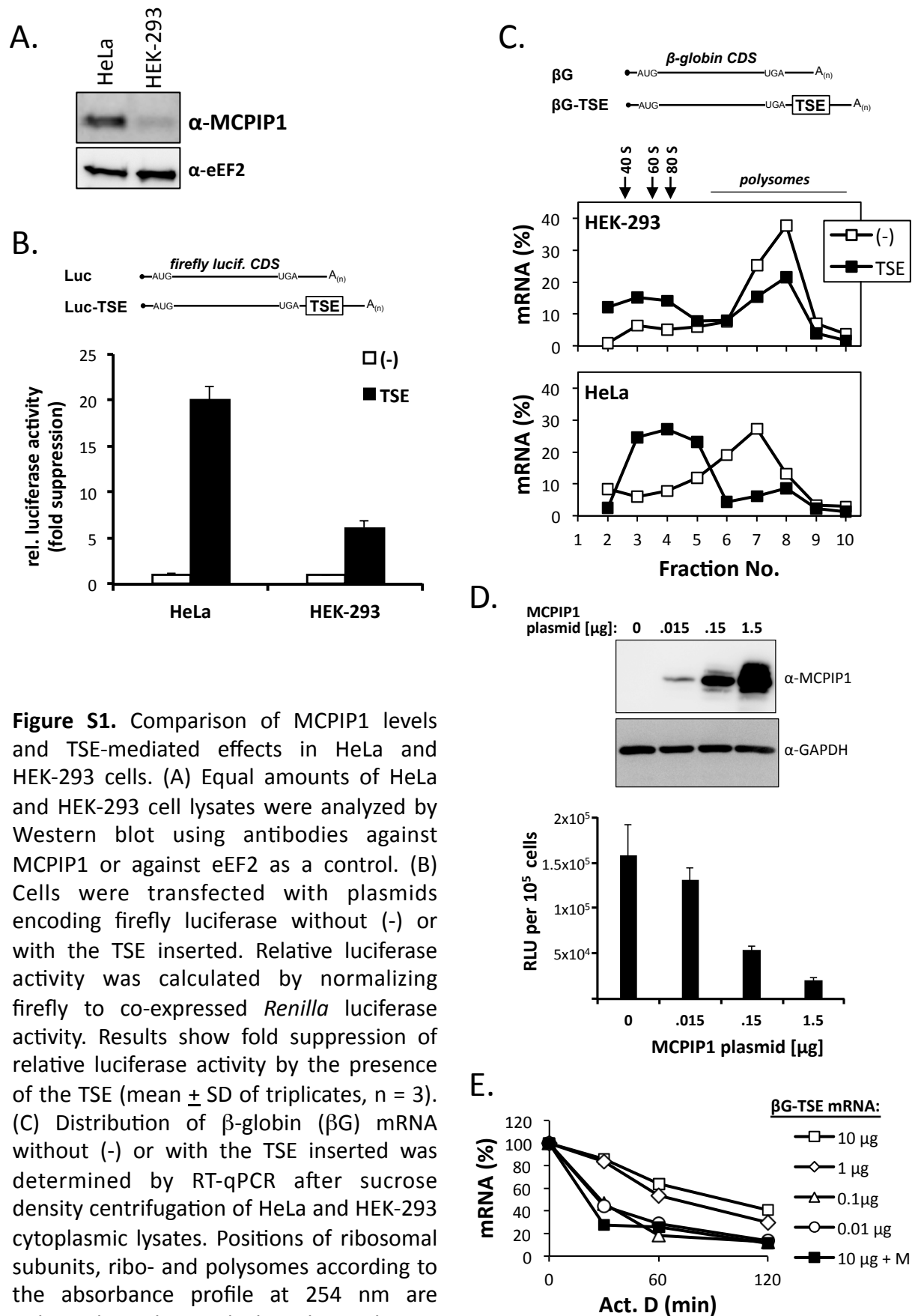


Figure S1. Comparison of MCPIP1 levels and TSE-mediated effects in HeLa and HEK-293 cells. (A) Equal amounts of HeLa and HEK-293 cell lysates were analyzed by Western blot using antibodies against MCPIP1 or against eEF2 as a control. (B) Cells were transfected with plasmids encoding firefly luciferase without (-) or with the TSE inserted. Relative luciferase activity was calculated by normalizing firefly to co-expressed *Renilla* luciferase activity. Results show fold suppression of relative luciferase activity by the presence of the TSE (mean $\pm$ SD of triplicates, $n = 3$). (C) Distribution of β -globin (β G) mRNA without (-) or with the TSE inserted was determined by RT-qPCR after sucrose density centrifugation of HeLa and HEK-293 cytoplasmic lysates. Positions of ribosomal subunits, ribo- and polysomes according to the absorbance profile at 254 nm are indicated. Similar results have been obtained in HEK-293 cells.

ned in three independent assays. (D) HEK-293 (4×10^5 cells per 9.6 cm^2 culture dish) were transfected with the indicated amounts of strep-MCPIP1 expression plasmid and with a firefly luciferase plasmid without insertions to monitor nonspecific suppression. Lysates were analyzed by Western blot for strep-MCPIP1 amounts and for firefly activity (relative light units, RLU, mean $\pm$ SD of triplicates for one out of two assays with similar results) to monitor non-specific suppression of expression. (E) HEK-293 cells were transfected with the indicated amount of β G-TSE plasmid per 56 cm^2 culture dish. Strep-MCPIP1 at an amount corresponding to $0.015 \text{ }\mu\text{g}$ plasmid in (D) was co-transfected where indicated (+M). After overnight culture actinomycin D (Act. D, $5 \text{ }\mu\text{g/ml}$) was added to stop transcription and total RNA was isolated at the indicated times thereafter. β G-TSE mRNA was quantitated by RT-qPCR and is expressed in percent of the amount at 0 min.

Comment:

Compared to HeLa cells, HEK-293 cells express less MCPIP1 protein (Fig. S1A) and mRNA (not shown). Furthermore, HEK-293 cells displayed weaker TSE-dependent silencing of reporter mRNAs, assessed in terms of suppression of luciferase activity (Fig. S1B) and of decreased ribosome occupancy according to polysome profile analysis (Fig. S1C). This suggested that functions of over-expressed MCPIP1 would be detected more sensitively in HEK-293 cells.

Transfecting the cells with increasing amounts of strep-MCPIP1 plasmid resulted in non-specific suppression of protein expression as detected by firefly luciferase activity derived from co-transfected control reporter plasmid (Fig. S1D) as well as in microscopically apparent loss of viability in the highest concentration (not shown). This is in line with previous reports on pro-apoptotic function of MCPIP1, including HEK-293 cells as targets [Zhou, 2006 #154]. For all subsequent experiments the lowest amount of strep-MCPIP1 plasmid was applied to avoid non-specific effects.

MCPIP1 overexpression was first tested for its effect on mRNA stability. The degradation kinetics of the β -globin-TSE reporter in HEK-293 cells was slowed down by increasing the amount of transfected reporter plasmid (Fig. S1E), which might indicate saturation of endogenous MCPIP1 by the reporter mRNA. Indeed, co-expression of strep-tagged MCPIP1 fully restored rapid degradation, demonstrating that MCPIP1 contributes to destabilization caused by the TSE.

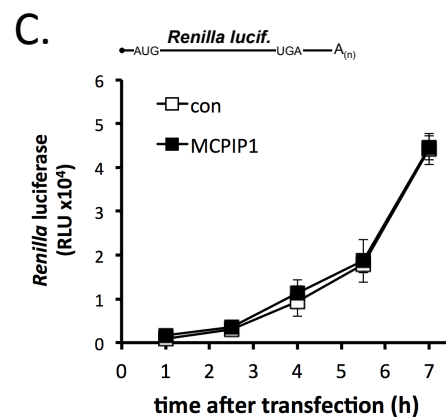
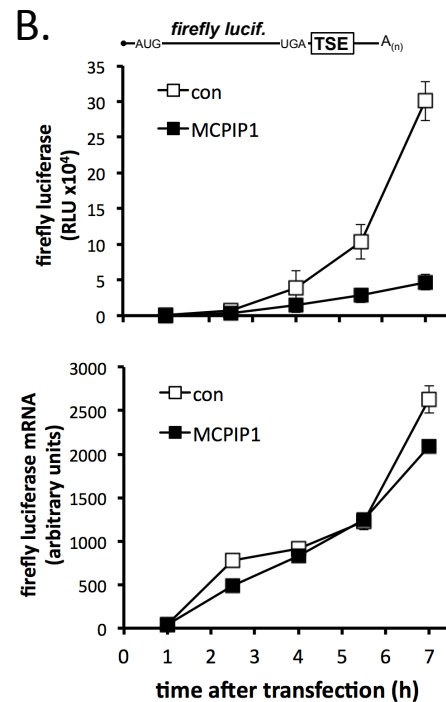
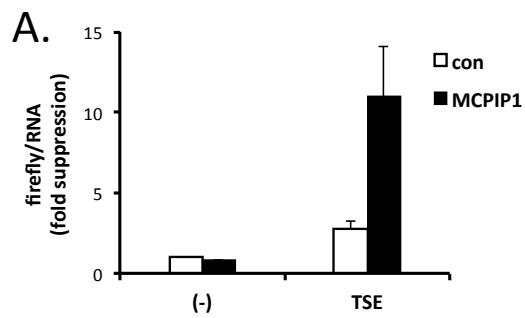


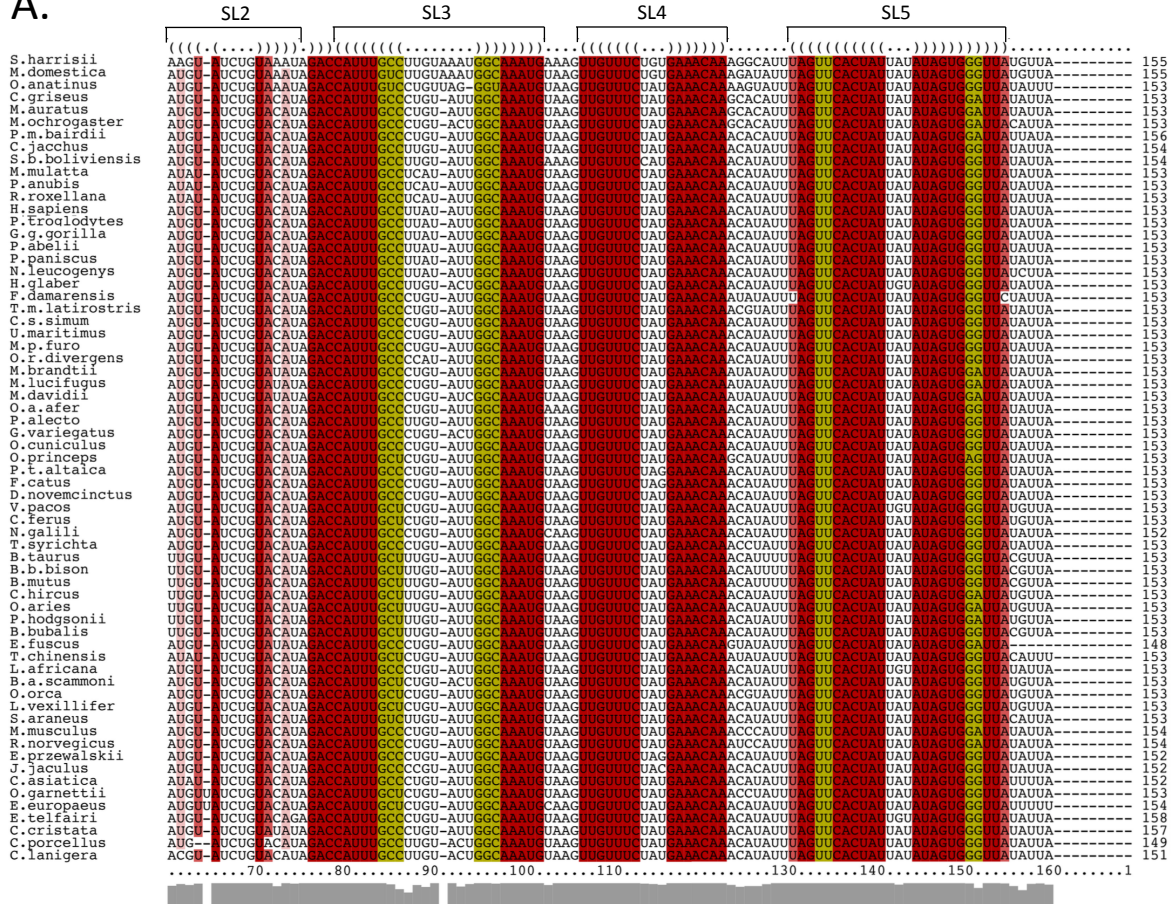
Figure S2. Time course of accumulation of luciferase activity and mRNA. HEK-293 cells were transfected with a firefly luciferase plasmid without (-) or with the TSE inserted and with a strep-MCPIP1 expression plasmid (MCPIP1) or empty vector (con). After overnight culture cells were lysed and firefly luciferase activity and mRNA quantified. Luciferase activities were normalized to the corresponding mRNA amount. Fold inhibition of normalized luciferase activity by the TSE and/or strep-MCPIP1 was calculated (mean $\pm$ SD, n=3, sample without TSE and strep-MCPIP1 expression set as 1). The results were confirmed in an assay using untagged MCPIP1 (not shown). (B,C) HEK-293 cells were first transfected with empty vector (con) or strep-MCPIP1 plasmid to ensure MCPIP1 expression at the time of transfection with reporter plasmids. The following day cells were re-transfected by the calcium precipitate method including firefly luciferase-TSE plasmid and *Renilla* luciferase plasmid (without TSE). Cells were lysed at the indicated times after removal of the calcium precipitate, followed by determination of luciferase activities (RLU, relative light units, mean $\pm$ SD of triplicates) and mRNA amounts (arbitrary units for firefly luciferase mRNA after normalization to *Renilla* mRNA, mean $\pm$ SD of duplicates).

Comment:

A lag in accumulation of firefly luciferase mRNA containing the TSE might occur in the presence of MCPIP1 and explain decreased accumulation of luciferase protein. That lag could have been missed at the late time point of determinations in Fig. S2A, but was ruled out by monitoring the kinetics of activity and mRNA after transfection (Fig. S2B): A strong suppression of firefly luciferase activity was evident (upper panel), whereas firefly luciferase mRNA levels were similar with and without ectopically expressed MCPIP1 at all time points (middle panel). Co-expressed MCPIP1 did not affect *Renilla* luciferase activity (Fig. S2C) or mRNA (not shown).

Supplemental Figure 3

A.



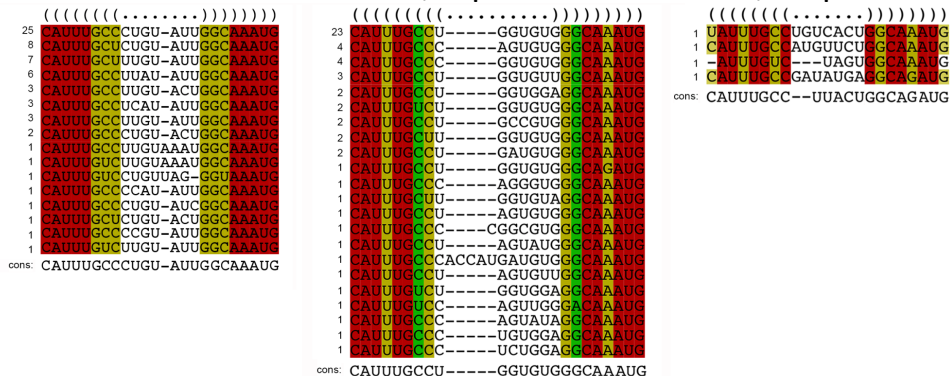
B.

SL3

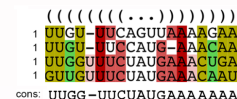
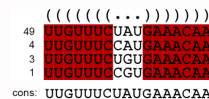
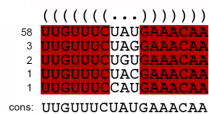
mammals

birds, reptiles

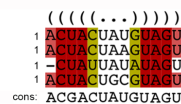
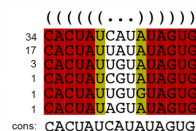
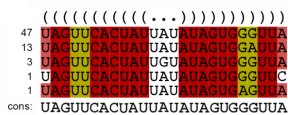
fish, amphibians



SL4



SL5



C.

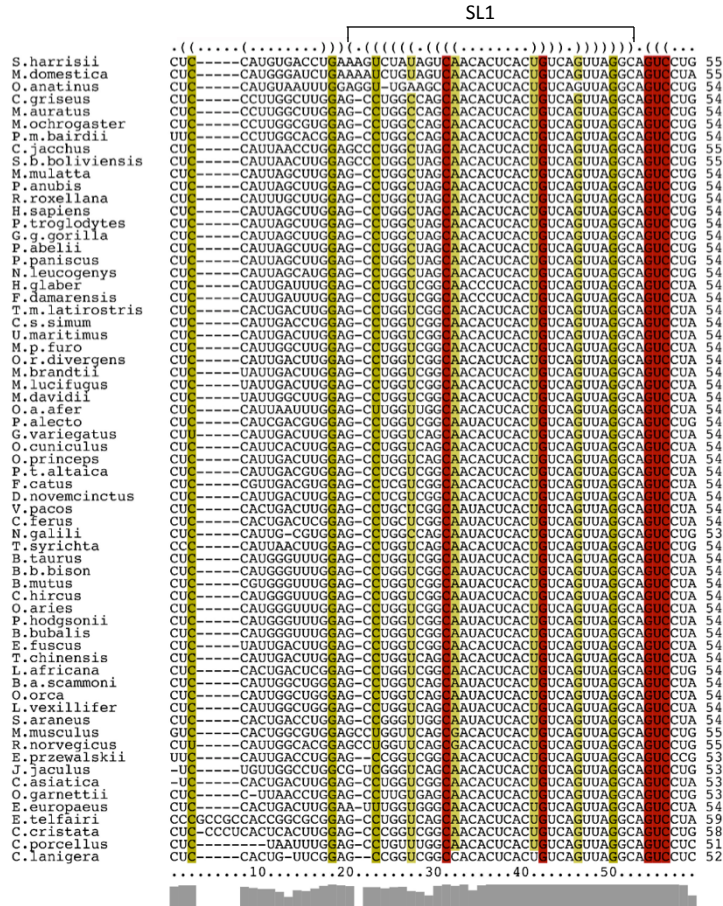


Figure S3. Sequence flexibilities of the TSE in vertebrates: (A) Sequence comparison of SL2, -3, -4 and -5 in mammals. (B) Conservation pattern of SL3, SL4 and SL5. For obtaining the conservation pattern, identical sequences of the stem loops out of the mLocARNA alignments of mammals, birds and reptiles as well as fish and amphibians were summarized. The number of species in which the respective sequence is present, is listed to the left of the sequence in the conservation pattern. The color annotation showing the conservation of base pairs was obtained from RNAalifold (see Figure 4A). (C) Sequence comparison of SL1 in mammals.

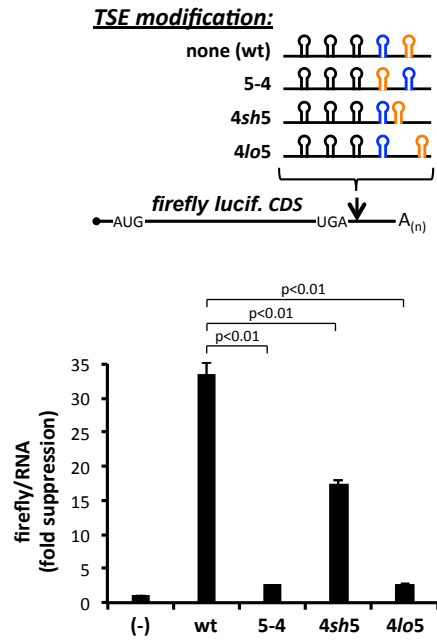


Figure S4. Influence of changes in the arrangement of SL4-5 on TSE function.

Hela cells were transfected with luciferase reporter constructs without insertion (-) or with the TSE containing SL4 and 5 without modification (wt), with positions of SL5 and SL4 exchanged (5-4), or with SL4 and SL5 separated by a shorter distance (shortened from 7 to 1 nt, 4sh5) or a longer distance (extended from 7 to 21 nt, 4lo5). Firefly luciferase activity was normalized to its mRNA determined in parallel. The change in the normalized luciferase activity was calculated as fold suppression compared to luciferase vector without insertion (mean $\pm$ SD of one out of three experiments with essentially similar results).

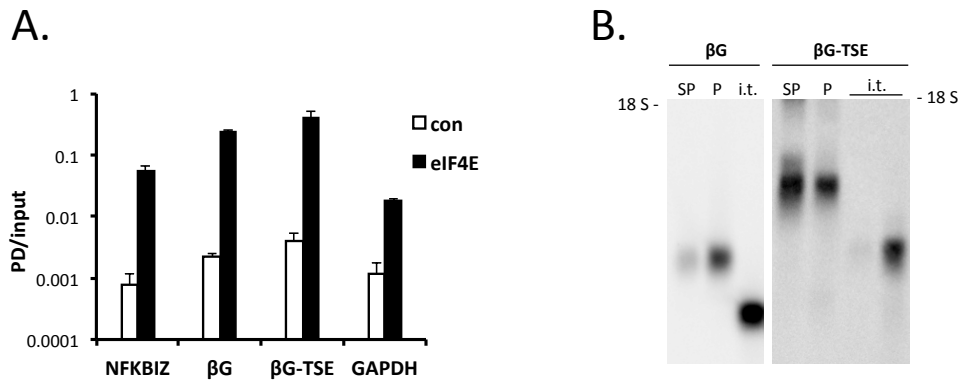


Figure S5. Co-purification of TSE-containing mRNAs with eIF4E and with polyadenylated RNA. (A) HeLa cell lysates were subjected to pulldowns with irrelevant (con) or eIF4E-specific antibodies. Co-precipitated endogenous mRNAs or plasmid-expressed β -globin mRNA without (β G) or with the TSE (β G-TSE) were quantitated by RT-qPCR. Results (mean $\pm$ SD, n=4) show the ratios of mRNA amounts in the pulldown material (PD) over amounts in the lysate (input). (B) HeLa cells were transfected with plasmids encoding β -globin mRNA without (β G) or with the TSE (β G-TSE). Polyadenylated RNA was isolated on oligo(dT) resin from pooled fractions after sucrose density centrifugation (SP, subpolysomal fractions 2-4; P, polysomal fractions 6-9) and analysed by Northern blot with a β -globin antisense probe. Note that the lower expression of β -globin-TSE mRNA compared to β -globin mRNA was compensated by applying higher amounts of RNA on the gel. *In vitro*-transcribed (i.t.) β -globin RNA without (400 pg) or with the TSE (150 and 400 pg) was loaded as non-adenylated size markers. The position of 18 S rRNA is indicated.

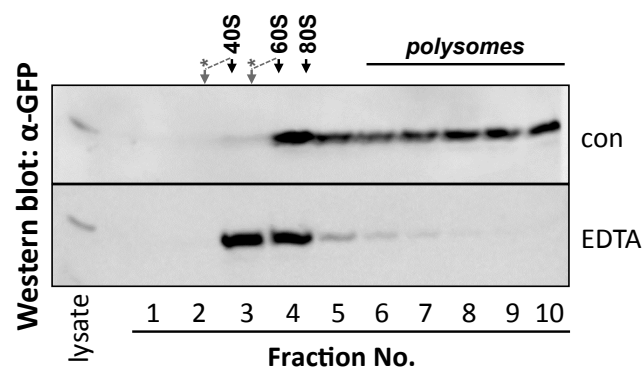
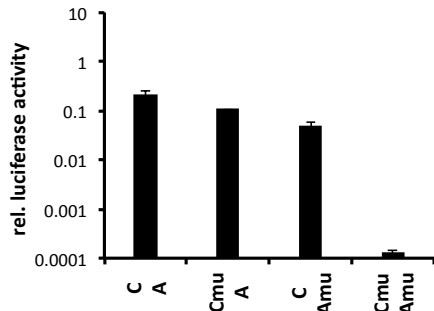
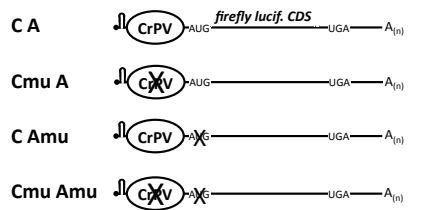


Figure S6. Co-sedimentation of GFP-tagged RPL10a with polysomes in sucrose gradients. HeLa cells were transfected with GFP-RPL10a plasmid. Two days later cytoplasmic lysate was prepared and centrifuged through sucrose gradients in buffer without EDTA (con) or without Mg^{2+} and with EDTA (30 mM) to dissociate ribosomes. Fractions were collected and protein precipitated with TCA. Equal volumes of redissolved protein were analyzed by Western blot with anti-GFP antibodies. Positions of ribosomal subunits, ribosomes and polysomes are indicated (*asterisks* denote positions of ribosomal subunits after reduced sedimentation due to EDTA (1)) .

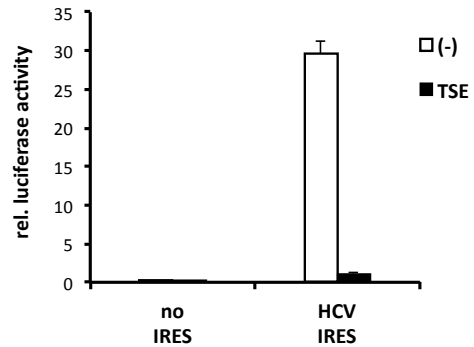
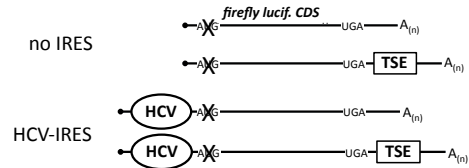
Reference

1. Blobel, G. (1971) Isolation of a 5S RNA-protein complex from mammalian ribosomes. *Proc. Natl. Acad. Sci. U. S. A.*, **68**, 1881-1885.

A.



B.



C.

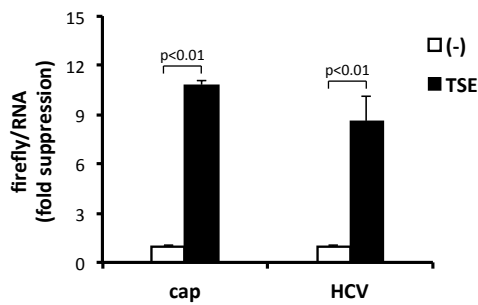


Figure S7. Effect of the TSE on IRES-dependent translation. (A) Plasmids containing a 5' hairpin structure followed by the CrPV IRES (C) or an inactive mutant (Cmu) and the luciferase coding sequence with intact (A) or mutated start codon (Amu, ATG→AAG) were co-transfected with *Renilla* luciferase plasmid into HeLa cells. Luciferase activities were determined the next day. Firefly luciferase activity was normalized to *Renilla* (mean $\pm$ SD of triplicates). (B) Plasmids containing firefly luciferase coding sequence with a mutated start codon without IRES or with the HCV IRES inserted upstream and without (-) or with the TSE inserted downstream were expressed in HeLa cells. Firefly activity was normalized to co-expressed *Renilla* activity as in (A). (C) Plasmids for cap-dependent and HCV-IRES-dependent expression of firefly luciferase without (-) or with the TSE inserted were transfected into HeLa cells. Results show fold suppression of firefly luciferase activity after normalization to the corresponding mRNA amount (values for plasmids without TSE set as 1, mean $\pm$ SD of duplicates).