Supplemental Figure Legends

Figure S1, related to Figure 1. **Mutation analysis of the *Rpl38* gene in *Ts, Tss*, *Rbt* and transgenic rescue with *Rpl38*.** (A) Physical map of the *Ts* and *Tss* chromosome region and position cloning of *Ts*. The *Ts* locus was mapped between markers *D11Rin56* and *D11CA2*. A BAC contig encompassing approximately 250kb of the *Ts* critical region was constructed between markers *D11Rin56* and *D11CA2*. These new markers were employed for fine mapping of the second *Tss* mutation, which was localized to an interval between the markers *D11CTT2* and *D11CA8*, spanning a 85kb DNA fragment. This mapping data suggested that *Ts* and *Tss* might be allelic. To further narrow down the critical region that contains the *Ts* locus, we undertook transgenic rescue with BAC DNAs, namely 261N17, 5464D10, and 256M20 for their capacity to rescue the *Ts* phenotype. DNA from clone 256M20 completely rescued the phenotype of *Ts*/+ mice, including skeletal patterning defects and embryonic lethality in homozygote embryos. *Rpl38* and Tweety homolog2 (*Thyh2*) were localized on BAC clone 256M20. We found no difference in the nucleotide sequence of the *Thyh2* cDNA clone between *Ts* and wild-type mice (data not shown). Their relative locations and the transcriptional orientations are depicted at the bottom. (B) Southern blot analysis of the *Rpl38* gene in mutant mice. Probe1 and exon4 of *Rpl38* were used as probes. *Ts/+* and *Tss/+* showed an extra band in addition to the wild-type band. All probes are depicted in Fig. 1C. (C) PCR analysis of the genome structure of the *Rpl38* locus in *Ts* mice, using primers, SP99 and SP102 selected outsides of all the exons and spanning a total of 19kb. The genomic DNA of *Ts/+* mouse yielded a 1kb band, but the genomic DNA of *+/+* mice did not. Primer positions are depicted in Fig. 1C. (D) RT-PCR analysis of the *Rpl38* transcripts from *Ts/+* and *Tss/+* mice. Note that the *Tss* mutation has a 375bp insertion between exon3 and exon4 in addition to the deletion of a base A in exon3 (data not shown). This is consistent with an extra band of 620bp that is detected in RT-PCR products amplified from *Tss/+* mice with the primers F1 and R1 and Northern blot analysis of *Tss*/+ mice that showed a larger 850b band in addition to the normal 420b band (Figure 1F). (E) Transgenesis with a *Rpl38* cDNA rescues the skeletal phenotypes of *Ts*/+ mice. Compare with *Ts/+* axial skeletal phenotypes shown in Figure 2. (F) Western Blot analysis for RPL38 and RPL4 in somite stage 40 WT, *Ts/+*, and *Ts/+;* pCAGGS-*RPL38* embryos. The decrease in RPL38 protein expression in *Ts*/+ parallels that of the mRNA (Figure 1F) and is restored to normal levels in *Ts/+;* pCAGGS-*RPL38* embryos. Expression of an additional RP, RPL4, is shown.

Figure S2, related to Figure 2. **Whole-mount in situ hybridization and qPCR analysis of Hox gene expression.** (A) No changes in the spatial expression boundaries of Hox genes are observed between Wt and *Ts*/+ embryos. (B) qPCR analysis of Hox gene expression from the neural tube and somites of Wt (n=3) and *Ts*/+ (n=3) embryos at 40 somite stage (~E11.0). No statistically significant differences in Hox gene expression are observed in *Ts*/+ embryos compared to Wt for the majority of Hox genes ($P > 0.05$), except for *Hoxb4* and *Hoxd4*. * P=.05, ** P=.025. Data are presented as the average ± SEM.

Figure S3, related to Figure 3. **Analysis of rRNA levels, rates of *de-novo* protein synthesis, and establishment of a microscale polysome profiling approach in *Ts*/+ embryos.** (A-B) Y10b immunofluoresence staining of somite stage 40 (~E11.0) Wt (A) and *Ts*/+ (B) transverse tissue sections at the forelimb level (10X). (C-D) Close-up of the neural tube (60X) of the same tissue sections shown in A-B. (E-F) Quantification of mean fluorescence intensity of Y10b staining within the neural tube (E) and somites (F) of Wt and *Ts*/+ tissue sections shown in A-B. (G-H) Relative S35 methionine incorporation to monitor the rates of *de-novo* protein synthesis in the neural and somites of stage 40 (~E11.0) Wt and *Ts*/+ embryos. (I) Representative polysome profile from neural tube and somite cellular extracts from E11.5 embryos, showing individual fractions collected on the bottom of the graph. (J) Ethidium bromide staining of rRNA species in individual fractions. Note the presence of both the 28S and 18S rRNA forms, showing the presence of mature ribosomes in each of these fractions. (K) β-actin mRNA was normalized to 18S rRNA by qPCR analysis. Note no difference in β -actin polysome distribution between Wt and *Ts*/+ is evident. Data are presented as the average ± SEM.

Figure S4, related to Figure 4. **Rescue of Hox gene protein expression in *Ts*/+; pCAGGS-*Rpl38* embryos, in-vitro translation of *Ts*/+ mRNA and stable knockdown of RPL38 in C3H/10T1/2 cells.** (A) Western blot for HOXA5 and HOXB13 protein levels in microdissected neural tube & somite tissue fragment from WT and *Ts*/+;pCAGSS-*Rpl38* embryos reveals complete rescue in protein levels. (B) Stable knockdown of RPL38 in C3H/10T1/2 cells. Western blot analysis for RPL38, HOXA5, and HOXC4 is shown. Note that RPL38 knockdown results in a decrease in HOXA5 protein levels, a target Hox mRNA found translationally deregulated in *Ts*/+ embryos, but not HOXC4. qPCR analysis of *Hoxa5* reveals no change in mRNA expression upon *Rpl38* shRNA knockdown. (C) Poly (A) mRNA was isolated from the neural tube and somites of *Ts*/+ embryos and *in vitro* translated (methods). GFP mRNA was *in vitro* translated as a control and Western Blot for HOXA5 is shown. (D) Representative relative [35S] methionine incorporation to monitor the rates of *de-novo* protein synthesis in the neural tube and somites of stage 40 embryos with distinct RP deficiencies (see also Figure 4P).

Figure S5, related to Figure 5. **Skeletal patterning in *P53*$^{+/-}$; *Ts*/+ mice and D-V neural patterning markers in *Ts*/+ embryos.** (A) Shown is the expression of Sonic Hedgehog (Shh), Pax6, Nkx2.2, and Pax7 in the spinal cord of stage-matched E11.5 WT and *Ts*/+ embryos, n=5 embryos were examined from each genotype. (B) Comparison of skeletal patterning in Wt, P53+/, and P53+/;*Ts*/+ newborn mice. No skeletal patterning defects are observed in Wt (top) and *P53*$^{+/-}$ (middle) mice. None of the axial skeletal patterning defects present in *Ts*/+ mice (see also Figure2) , such as the formation of an extra rib (number 14) and the asymmetric attachment of eight pairs of vertebrosternal ribs are rescued in *P53*$^{+/-}$; *Ts*/+ mice (bottom).

Figure S6, related to Figure 6. **Characterization of HoxA5 induction following**

Retinoic Acid (RA) treatment of EC cells and sucrose gradient fraction of Hox mRNAs. (A) Accumulation of Hox mRNAs in fraction 3 (non-ribosomal) of sucrose gradients, in *Ts*/+ embryos compared to WT. This accumulation of Hox mRNAs not bound by ribosomes in *Ts*/+ embryos is consistent with the decrease in 80S-mRNA complex formation that is also observed (Figure 6A). (B) qPCR analysis for a representative Hox gene, *Hoxa5*, in Human EC cells is shown following treatment with RA. Time is indicated in hours (Hrs).

TableS1. **Sequences of Hox genes oligonucleotides used in polysomal qPCR analysis.** All oligonucleotides are HPLC purified.

TableS2. **Sequences of ribosomal protein oligonucleotides used in a ribosomal protein expression screen.** All oligonucleotides are HPLC purified.

TableS3. **Ribosomal Protein expression patterns during organogenesis**. Each column is a tissue or cell type; each row is ribosomal protein gene. Shown are the expression values in $\log_2$ space.

EXTENDED EXPERIMENTAL PROCEDURES

Generation of transgenic mouse lines and rescue of the *Ts/+* phenotype. In transgenesis with DNA of BAC clones, the supercoiled form was used. As a vector construct, cDNA of the *Rpl38* gene without 5' and 3'UTR sequences was inserted into the pCAGGS expression vector (Niwa et al., 1990) that consists of CMV-IE enhancer, Chicken b-actin promoter and Rabbit b-globin polyA signal. For microinjection, DNAs of BAC clone 256M20 and the plasmid pCAGGS-*Rpl38* were prepared by alkaline lysis and cesium chloride gradient ultracentrifugation. Extracted DNAs were extensively dialyzed into TE. Isolated DNAs were injected at a concentration of 1ng/μl with injection buffer, 10mM Tris-HCl (pH 7.5), 0.1mM EDTA, and 100mM NaCl, into the fertilized mouse oocytes isolated from strain C57BL/6J. BAC transgene (tg) carriers were identified in PCR assay for both the T7 and SP6 arms, and were crossed to (TSJ/Le-*Ts/+* x C57BL/6J) F1 mice. The resultant progeny was examined for *Ts/+* phenotype after genotyping for the transgene and *Ts* mutant allele. The progeny carrying both the transgene and the *Ts* mutation were intercrossed, and the resultant progeny were examined for *Ts* phenotype after genotyping of the transgene and the *Ts* mutation. The pCAGGS-*Rpl38* transgene carriers were identified by PCR utilizing vector specific primers, and were crossed to (TSJ/Le-*Ts/+* x C57BL/6J) F1 mice. Similar to BAC DNA transgenesis, the progeny was examined for the *Ts* phenotypes.

cDNA selection. Total RNA was extracted from whole embryos of the C57BL/6J strain with ISOGEN (Nippon Gene Ltd., Japan) and the mRNA was purified using Quickprep Micro mRNA Purfication Kit (Amersham Pharmacia Biotech). First strand and second strand DNA were synthesized by ZAP-cDNA Synthesis Kit (Stratagene) and after blunting the cDNA ends, ERI adaptor (TAKARA Ltd., Japan) was ligated for PCR amplification. For prehybridization, we used mouse COT1 DNA (Gibco). Hybridization and other steps were described previously(Akiyama et al., 1997).

***Rpl38* Mutation analysis.** Primers (Rpl38F, 5'-CGCCATGCCTCGGAAAATTG-3'; Rpl38R, 5'-ATAGTCACACGCAGAGGGCT-3') were used for RT-PCR amplification of Rpl38 cDNA from *Tss* and *Rbt* mutant mice. RT-PCR products were sequenced directly. This amplified product was also used for Southern and Northern blot analyses. Primers (SP99, 5'-TTCTCACAGATGTCTCGCTAG-3'; SP102, 5'-AACCTCCATTTCCAGCCAGG-3') were used to amplify the genomic DNA encompassing Rpl38. Primers (PRF3, 5'-CGTACTCAGTTTCTGTT-3'; RPR2, 5'-GCCATAACCTGCTGTTCCGA-3') were used to amplify the genome DNA encompassing intron 2 to exon 3 of *Rpl38*. PCR-products amplified by primer pairs (5'-GAGGATTCCTAGACAATCCT-3', 5'- CACGTTAGGTGCCAAACTCG-3' and 5'-GATTCTGCACCAGTTCTCATCC-3', 5'-TAATGCTGCCCATGCCACT-3') were used as probes of Probe1 and Exon4 in Southern blot analysis. The reaction conditions were as follows: denaturation at 94 ˚C for 1 min, annealing at 55˚C for 1 min, and extension at 72 ˚C for 1 min, for 35 cycles.

Dissection of Somites and Neural Tube. WT and *Ts*/+ embryos were staged precisely by counting the number of somites. Embryos at somite stage 40 (~E 11.0) were employed for the majority of studies. All dissections were performed in media (DMEM F12 1:1, 10% FBS, and 1% penn-strep) in a Sylgard dissection dish (Sylgard 184 Silicone Elastomer Kit; Dow Corning). Embryos were pinned to the dish (Austerlitz dissecting pins, FST) with the ventral surface of the embryo facing down. The neural tube and somites were microdissected utilizing a 5mm depth 15° blade (Sharpoint) starting at the level of the first somite, which served as a landmark, to the very tip of the tail. Somites and neural tube were then transferred to Hank's balanced salt solution, Ca^{2+} and Mg^{++} free, containing 1% trypsin and incubated at 37°C for 45 minutes. After incubation, trypsin solution was removed and the tissues were resuspended in 1 ml of media (DMEM F12 1:1, 10% FBS, and 1% penn-strep) and thoroughly mixed to disperse cell aggregates. Cells were then spun down at 1,500 rpm for 5 min and washed twice with PBS. For some experiments, cells were directly resuspended in TRIzol Reagent (Invitrogen) and were either stored at -80°C or RNA was directly extracted according to the manufacturer's protocol.

Whole-mount *in situ* hybridization. Whole-mount *in situ* hybridization was carried out using digoxygenin-labeled antisense RNA probes as previously described (Barna et al., 2000). However, the last post-antibody wash was carried out overnight at 4°C.

Section *in situ* hybridization. We carried out *in situ* hybridization using digoxygenin-labeled antisense RNA probe, on 10 mm tissue sections, which was synthesized from the full coding sequence (1-213 bp) of *Rpl38*, as described (Holmes and Niswander, 2001). The sections were incubated in BM Purple staining solution overnight at 4°C, followed by an addition 3 days at room temperature. The staining solution was changed every other day.

Skeletal Staining. Alcian-blue and alizarin-red staining of cartilage and bone were performed as previously described (Barna et al., 2000).

qPCR analysis. RNA was isolated using manufacture's protocol supplied with TRIzol Reagent (Invitrogen). For ribosomal protein profiling and Hox gene expression, 1 mg of RNA was Dnase-treated with TURBO DNA-*free* kit (Ambion). 8 ml of Dnase-treated RNA was further converted to cDNA with SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen), and 1 ml of cDNA was used to run a SYBR green detection QPCR assay (Stratagene QPCR system) in a 50ml reaction containing 25 ml SYBR Green PCR Master Mix (Applied Biosystems). All primers were used at 300nM concentration per reaction.

Ribosomal protein expression profiling. The following mouse tissues – lung, limb, liver, heart, kidney, pancreas, somites, eye, spleen, stomach, and thymus – were micro-dissected from WT embryos at E11.5. The pancreas was dissected from WT embryos at E13.5. All dissections were performed in media (DMEM F12 1:1, 10%

FBS, and 1% penn-strep) in a Sylgard dissection dish (Sylgard 184 Silicone Elastomer Kit; Dow Corning). Murine embryonic fibroblasts were obtained from E13.5 embryos and cultured in D-MEM High Glucose (4.5g/L Glucose, 0.584g/L L-Glutamine, 3.7g/L $NaHCO_3$, phenol red) with 10% FBS and 1% Penn-strep. Neural stem cells (see experimental procedures) were cultured in media containing 50% D-MEM/F-12 1:1 (GIBCO 11039), 50% Neurobasal media (GIBCO 21103), 1% N-2 Supplement (GIBCO 0723), 1% B-27 Supplement (GIBCO 0778), 0.067% BSA Fraction V (GIBCO 15260), 0.01% recombinant Murine EGF (PetroTech), 0.01% recombinant Murine FGF-basic (PetroTech), and 1% Penn-strep. Early passage ES cell lines were grown in the absence of a feeder layer on gelatinized plates in DMEM containing 15% FCS, leukemia-inhibiting factor, 1% Penn-strep, L-glutamine, and non-essential amino acids. RNA was isolated using manufacture's protocol supplied with TRIzol Reagent (Invitrogen). Tissues were homogenized directly in TRIzol (Invitrogen). In all qPCR assays (see experimental procedures), ribosomal protein primers were used at 300nM concentration per reaction (Table S2). The clustering of ribosomal protein expression in different embryonic tissues was generated and visualized using Cluster 3.0 (Eisen et al., 1998) and Java TreeView (Saldanha, 2004) software.

Neural stem cell derivation. ES cells were differentiated into pan-neural precursor cells through embryoid body formation for 4 days and selection in ITSFn media for 5-7 days, and they were maintained in FGF2 and EGF2 (PeproTech) as previously described (Conti et al., 2005).

Retinoic Acid (RA) treatment of EC cells. Human EC cells, NTERA-2 clone D1, (ATCC) were maintained at high density in DMEM media supplemented with 10% fetal calf serum. Cultures were treated with all-trans RA (Sigma) at a final concentration of 10um as previously described (Simeone et al., 1991). Cells were re-fed every 48 Hrs with fresh medium containing RA.

Protein extraction and Western Blot analysis. Cells from microdissected somites and neural tube were lysed in protein lysis buffer [400mM NaCl, 10mM Hepes pH 7.9, 0.1 mM EGTA, 0.5mM PMSF, 1% Triton, 0.5mM DTT, 5% Glycerol] for 30 minutes on ice and cells were vortexed every 10 minutes. Cell lysates were spun at 15000 rpm for 10 min at 4°C to remove debris and supernatant was collected. For western blot analysis, 20 μg protein extracts were employed. The membranes [Bio-Rad] were blocked in 5% nonfat dry milk in PBST for 1 h, and incubated overnight at 4°C with anti-Hoxa5 (Abcam) and anti-Hoxb13 (Aviva Systems Biology), or for 1h at room temperature with anti-Hoxa11 (Aviva Systems Biology) and anti-Hoxc4 (Covance) antibodies, washed 3 times for 20 min in PBST, incubated with appropriate secondary antibodies conjugated to horseradish peroxidase (GE Healthcare) for 45-1h, and washed 3 times for 20 min in PBST. The western-blot signals were developed using ECL detection reagents (GE Healthcare).

Immunofluorescence. Embryos were dissected and fixed for 1h at 4°C in 4% PFA in PBS, washed with PBST for 15 min and then washed with PBS for 15 min, 3 times at 4°C. Embryos were then equilibrated in 30 % sucrose in 0.1M potassium buffer overnight at 4°C, transferred to cold O.C.T. Compound (Tissue-Tek), incubated on ice for 1h, oriented and froze down on dry ice. Embedded embryos were sectioned at 10 mm; sections stored at -80°C. Before the immunofluorescence procedure, sections were equilibrated to RT and rehydrated in blocking buffer (1% heat-inactivated goat serum, 0.1% Triton X-100 in PBS) for 1h at RT. The sections were then incubated with anti-Hoxa5 antibody (kind gift of Dr. Lucie Jeannotte, Centre de recherché en cancérologie de l'Université Laval, Canada) or anti-ribosomal RNA 5.8s antibody (Abcam) or PEA3 1:100 (Santa Cruz), or Shh, Nkx2.2, Pax6, Pax7, Isl1 (Developmental Studies Hybridoma Bank) in blocking buffer overnight at 4°C, washed with blocking buffer 3 times, 20 min each and incubated with appropriate 488-fluorescent secondary antibodies (Invitrogen) for 1h at RT, protected from light. Slides were washed with blocking buffer 3 times, 20 min again and then mounted with Vectashield Mounting Medium with Propidium Iodide (Vector Laboratories).

Polysome fractionation and RNA/Protein isolation. For each polysome set, somites and neural tube from three WT and three *Ts*/+ 40-somite stage embryos were employed (see Dissection of Somites and Neural Tube). A minimum of four polysome sets (n=4) per genotype was used for polysome profiling analysis (See Figure 4). Cells were resuspended in PBS containing 100 mg/ml cycloheximide, and incubated on ice for 10 min. Cells were centrifuged at 1,500 RPM for 5 minutes at 4°C and resuspended in 1 ml of lysis buffer (10mM Tris-Hcl pH8, 150mM NaCl, 10 mM $MgCl_2$, 0.25% NP-40, 0.1% Triton X-100, 0.1 U/ml Rnasin, 200 μg/ml cycloheximide, 20mM DTT). Samples were incubated on ice for 30 min and vortexed every 10 min. The sample was centrifuged at 10,000 rpm for 5 min at 4°C and the supernatant was carefully isolated and loaded onto 10-50% sucrose gradients containing 0.1 mg/ml heparin and 2mM DTT and centrifuged at 37,000 rpm for 2.5 h at 4°C (SW40 rotor). The sucrose gradient was subsequently fractionated with a gradient fractionation system (ISCO) connected to a UV detector to monitoring absorbance at 252nm. As small embryonic tissue fragments were employed, a limiting factor was the analog output of absorbance that fell below the range that could be accurately recorded with a chart recorder. We therefore employed a digital voltmeter that was attached to the spectrometer to digitally record the full range of data as that enabled better resolution of the polysome profile. RNA was extracted from all fractions and resolved in denaturing agarose-formaldehyde gel to visualize 18S and 28S RNA representing the 40S and 60S subunits, respectively. The polysomes fractions were determined to be #9 through #13 and these were employed for subsequent qPCR analysis (see Figure S6). 1.5 ml of TRIzol Reagent (Invitrogen) was added to each collected fraction; samples were incubated at RT for 5 min and stored at -80°C. RNA was isolated from polysomal fractions using the PureLink RNA Mini Kit (Invitrogen). Each fraction was eluted in 22 μl of DEPC

water, and then Dnase-treated with TURBO DNA-*free* kit (Ambion). 8 μl of Dnase-treated RNA was reverse-transcribed using SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen). The resulting 20 μl of cDNA were diluted with 10 μl of water, and 1 μl of cDNA per reaction was used for QPCR analysis with the SYBR Green PCR Master Mix (Applied Biosystems). All Hox primers (Table S1) were used at 300nM concentration per reaction and b-actin primers (Forward: 5'-TTGCCGACAGGATGCAGAA-3'; Reverse: 5'-GCCGATCCACACGGAGTACT-3') were employed for the purpose of normalization. b-actin mRNA was normalized to 18S rRNA (Forward:5'-AAACGGCTACCACATCCAAG-3'; Reverse:5'-TACAGGGC CTCGAAAGAGTC-3') by qPCR analysis and no difference in the absolute amount of b-actin in polysomes was evident between Wt and *Ts*/+ samples. Polysome dissociation was achieved by the addition of 1mM Puromycin to lysis buffer lacking cycloheximide as previously described(Blobel and Sabatini, 1971). In brief, following lysis the samples were incubated at 37°C for 15 minutes. Lysates were loaded onto a 10-40% sucrose gradient to facilitate ribosomal subunit separation and fractionated as described above. Following fractionation, proteins were isolated using the ProteoExtract Protein Precipitation Kit (EMD Biosciences), and western-blotted as above with anti-Rpl38 (kind gift of Dr. Cristina Al-Khalili Szigyarto, Royal Institute of Technology/AlbaNova, Sweden), anti-Rpl4 (Santa Cruz Biotechnology), anti-Rps6 (Cell Signaling), anti-Rps3 (Abcam), or anti-Rpl10a (Santa Cruz Biotechnology).

Ribosome Fractionation. Ribosome and nonribosomal fractions were collected essentially as described (Mazumder et al., 2003). In brief, neural stem cells or NTERA-2 cl.D1 cells (1.2×10^8) were treated with 100ug/ml of cycloheximide for 1 minute at 37°C. The cells were subsequently removed from the incubator and washed with 10ml of ice-cold PBS containing 100ug/ml of cycloheximide. The cells were lysed in 1.5 ml lysis buffer containing 20mM Tris (pH 7.4), 10mM $MgCl_2$, 300mM KCL, 10mM DTT, 100units/ml RNasin, 1% Triton, 100ug/ml cycloheximide. Cells were incubated for 10 minutes at 4°C on a rotator. After centrifugation at 15,000 x g for 10 minutes at 4°C to clarify the lysate, the supernatant was layered over a sucrose (20% w/v) cushion containing cycloheximide and centrifuged at 70,000 RPM, 4°C in a TLA-110 ultracentrifuge rotor for 4 hours. To remove any ribosome contaminants, the supernatant was subjected to a second centrifugation at 70,000 RPM for 2 hours. RNA was extracted from both fractions by Trizol and resolved in denaturing agarose-formaldehyde gel. The gel was stained with ethidium bromide to visualize rRNA. For immunoblot analysis, ribosome pellets were directly resuspended in Laemmli's buffer and cytosolic fractions were precipitated, concentrated utilizing the ProteoExtract Protein Precipitation Kit (EMD Biosciences), and resuspended in Laemmli's buffer. Proteins from ribosomal and nonribosomal fractions were subjected to Western Blot analysis with monoclonal anti-Rpl4 (Santa Cruz Biotechnology), anti-Rpl5 (Abcam), and anti-Rpl38 (kind gift of Dr. Cristina Al-Khalili Szigyarto, Royal Institute of Technology/AlbaNova, Sweden) antibodies overnight at 4°C.

[^{35}S] Labeling. Somites and neural tube from WT and *Ts*/+ 40-somite stage embryos were employed (see Dissection of Somites and Neural Tube). 1.5x10^{6} cells were serum starved in methionine-free DMEM (Invitrogen) for 1hour at 37^{o}C. Following starvation, 42 μCi of [^{35}S]-labeled methionine as well as dialyzed FBS were added to each sample and incubated at 37^{o}C for an additional hour. Cells were prepared using a standard protein lysate protocol. The cell lysates were resolved on a 10% SDS polyacrylamide gel and transferred onto a PVDF membrane (Biorad). The membrane was exposed to autoradiography film (Denville) for 24 hours and developed. Actin (Sigma) was used as a loading control.

Fluc and Rluc Activity. The neural tube and somites from somite stage 40 CMV-HCV-IRES^{T} and *Ts*/+; CMV-HCV-IRES^{T} embryos were microdissected (Dissection of Somites and Neural Tube). Cells were directly resuspended in 100ul of Passive Lysis Buffer (Promega) for 15 minutes at RT with gentle rocking. Firefly and Renilla luciferase activity was determined using the Dual-Luciferase kit (Promega) using a single tube luminometer (Optocomp1, MGM Instruments).

***In vitro* translation.** Poly(A) mRNA was isolated from the neural tube and somites of Ts/+ embryos using the Poly (A) purist-MAG kit (Ambion). *In vitro* translation reactions were performed using the Pierce Human *in vitro* Protein Expression Kit for mRNA Templates (Thermo). Reactions were analyzed by western blot as described above. GFP mRNA was used as a control.

C3H/10T1/2 *Rpl38* ShRNA knockdown. For shRNA knockdown, we employed the MISSION pLentiviral mediated gene-specific short hairpin RNA (shRNA) system(Moffat et al., 2006). Control and *Rpl38*-specific shRNA knockdown constructs were purchased from Sigma. ShRNA constructs were first transfected in 293 cells using the Lipofectamine 2000 Transfection Reagent (Invitrogen) to produce high-titer lentiviruses. C3H/10T1/2 cells were subsequently infected and Puromycin selection was carried out at a concentration of 5ug/mL to achieve stable knockdown.

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