

Supplementary Information

The following supplementary information is included as supplementary material tables and figures: supplementary material Table S1, description of mass spectrometry candidates identified in DLX- bound complexes from *Evf2*^{+/+} and *Evf2*^{TS/TS} nuclear extract lysates, supplementary material Table S2, summary of screen of transcribed ultraconserved sequences, supplementary material Table S3, description of *in vitro* transcribed RNAs, used in remodeling and ATPase inhibition and REMSA's. Supplementary material Fig. S1, intensity measurements of Evf2 clouds and BRG1 protein colocalization, supplementary material Fig. S2, map and expression of specific ultraconserved RNAs in E13.5 GE, supplementary material Fig. S3, additional REMSA's showing BRG1 promiscuous RNA binding properties.

Table Legends

Supplementary Table 1. Mass spectrometry identifies DLX-associated proteins in E13.5 GE nuclear extracts from *Evf2*^{+/+} (Top) and *Evf2*^{TS/TS} (Bottom) mice. Page 1: Top: The total number of proteins from *Evf2*^{+/+} is 87 (after removal of contaminants keratin, trypsinogen, and bovine serum albumin). 79/87 proteins are found in *Evf2*^{+/+}, but not *Evf2*^{TS/TS}, and therefore depend on the presence of *Evf2*. 32/79 *Evf2*-dependent proteins fall into one of the categories listed in the Table: chromatin remodelers (8, highlighted in yellow), chromatin binding (2), transcription factors (6), RNA helicases (7), splicing (3), polyadenylation (1), hnRNPs (5). Page 2: Bottom: The total number of proteins from *Evf2*^{TS/TS} is 15

(after removal of contaminants keratin, trypsinogen, and bovine serum albumin). 7/15 proteins are found in *Evf2^{TS/TS}*, but not *Evf2^{+/+}*. Proteins (8/15) found in both *Evf2^{TS/TS}* and *Evf2^{+/+}* are highlighted in green. DLX complexes isolated from *Evf2^{TS/TS}* nuclear extracts do not contain chromatin remodelers, RNA splicing or polyadenylation factors. DLX1 is present in complexes isolated from both *Evf2^{+/+}* and *Evf2^{TS/TS}*. Page 2: complete list of DLX bound proteins in *Evf2^{+/+}* nuclear extracts.

Supplementary Table 2. 16 ultraconserved RNAs expressed as opposite strand/ antisense transcripts were tested for expression in E13.5 GE by RT-PCR, and a subset were subcloned into pGEM, and verified by sequencing. The yellow highlights indicate RT-PCR detection in E13.5 GE, while green indicates verification by sequencing. 3/16 (*Dlx1UR*, *Ptc1UR* and *ShhUR*) are predicted to form the 3F secondary structure found in the *Evf2* 5' end.

Supplementary Table 3. Description of RNAs (*Evf2*, *Dlx1UR*, *28S*, *pGEM*, *tRNA*, and *ribihomopolymers*) used in remodeling inhibition and REMSA's. The sequence junctions between plasmid (blue, pGEM or pcDNA) and inserts (black) lengths, as well as the restriction enzyme for linearization are included in the schematic.

Figure Legends

Supplementary Figure 1. Intensity plots of RNA/protein clouds in E13.5 ganglionic eminence. *Evf2* fluorescent RNA in situ hybridization (FISH) and immunofluorescence detection of BRG1 protein is visualized by confocal

microscopy, quantified, and graphed. *Evf2* RNA FISH (green) and BRG1 protein (red), nuclei (DAPI, blue). Plots from 24 nuclei are shown.

Supplementary Figure 2. A database screen for ultraconserved RNAs expressed in E13.5 GE. In 2005, a database listing sequences of 1373 ultraconserved regions (UCRs) in the mouse genome (Woolfe et al., 2005). Mouse brain EST databases were screened for UCR sequences. 119 corresponding ESTs were identified, and verified for expression in E13.5 GE. The relative positioning of these 119 ultraconserved transcripts (ucRNAs) and coding exons was determined. **A.** Shows the percentage of ucRNAs in each category, with 16/119 expressed as opposite strand (OS) or antisense transcripts. **B.** A list of the 119 ucRNAs: the CR numbers are identifiers of those listed in the (Woolfe et al., 2005)UCR database. **C.** Schematic of novel ultraconserved lncRNAs expressed in E13.5 GE expressed as opposite strand/antisense transcripts in E13.5 GE identifies *Dlx1UR*, *Ptc1UR*, and *ShhUR*. ucRNA transcripts (blue) and protein coding exons (yellow). **D.** E13.5 GE qualitative RT-PCR identifies transcripts for ucRNAs: *Evf2*, *Dlx1UR*, *Ptc1UR*, and *ShhUR*. β -actin is also shown. PCR products generated when cDNA reactions were performed in the presence of reverse transcriptase (RT+), and absence (RT-) are shown.

Supplementary Figure 3. *Evf2*/BRG1 binding is promiscuous, and can be competed by 28S, *Dlx1/2UR*, and pGEM RNAs. **A.** RNA-fold analysis of the *Evf2*- 5' end (1-385). The region between 117-385 was shown to be necessary

and sufficient for Evf2 transcriptional activity in neural cell lines (Feng et al., 2006). The red box outlines the ultraconserved sequence. The blue box outlines a region (115 nt) of 100% identity between human, rat and mouse Evf2 sequence. Within the blue boxed region, a stem-loop structure (3F) is predicted by RNA-fold analysis. This 115 nt region of Evf2 is used for REMSA's. **B.** *Evf2* IR labeled probe is incubated with unlabeled RNAs in increasing concentrations (triangle: 7.5, 15, 30 picomoles). All lanes contain *Evf2* RNA IR labeled probe. Lanes: 1. (-) *Evf2* probe alone, 2-8. + BRG1, 3-5. + *Evf2* RNA (7.5, 15, 30 picomoles), 6-8. + *pGEM* RNA (7.5, 15, 30 picomoles), 9. (-) *Evf2* probe alone, 10-16. + BRG1, 11-13. + *Dlx1UR* RNA (7.5, 15, 30 picomoles), 14-16. + *28S* RNA (7.5, 15, 30 picomoles).

Supplementary Methods

Nuclear extract

E13.5 ganglionic eminences were harvested from *Evf2*^{+/+} or *Evf2*^{TS/TS}, or Swiss-Webster timed pregnant dams (Taconic), and the tissue flash frozen and stored at -80°C. Nuclear extracts were prepared from ganglionic eminences from ~10 embryos as described (Dignam et al., 1983) with the following modifications: tissue was incubated for 15 minutes on ice in 2.5% Igepal CA-630 in buffer A, and pellets were washed three times with 5% Igepal in buffer A before resuspension in 150 µl buffer C. Protein concentration was determined by the BCA Protein Assay (Pierce).

Co-immunoprecipitation

100 μ g of nuclear extract from E13.5 ganglionic eminences was diluted in Binding Buffer (25 mM HEPES-KOH pH 7.5, 150 mM NaCl, 5 mM MgCl₂, 0.1 mM EDTA, 0.1 % Triton X-100, 1mM DTT, 50 μ g/mL BSA and protease inhibitors) and pre-cleared with 50 μ l Protein G Agarose Beads (Roche) at 4° C for 1 hour with rotation. Beads were removed by centrifugation and the extract was pre-cleared with 50 μ l rabbit IgG-conjugated beads at 4°C for 1 hour with rotation. 5 μ g of anti-DLX (Feng et al., 2006) antibody, anti-BRG1 (Wang et al., 1996) antibody or rabbit IgG was added to the pre-cleared extract and incubated at 4°C overnight. 50 μ l of Protein G Agarose Beads (Roche) blocked with 10mg/ml BSA were added and incubated at 4°C for 1 hour. The beads were washed three times with Binding Buffer. 20 μ l of 1X SDS Sample Buffer were added to the beads for elution at 95° C for 5 min. Samples were analyzed by Western, probing with anti-BRG1 antibody (Wang et al., 1996), and detected using chemiluminescence.

Differential mass spectrometry

Column preparation

Rabbit *dll* antibody(Feng et al., 2006) or bovine serum albumin were crosslinked to cyanogen-bromide activated Sepharose 4B (GE Healthcare).

Immunoprecipitation

All buffers contained 0.5 mM DTT. Anti-*dll* and BSA columns were washed with 5 ml wash buffer (14 mM HEPES, 0.75 mM MgCl₂, 130 mM NaCl, 20 mM KCl, and 0.18 mM EDTA, pH7.9). 3.75 mg nuclear extract purified from wildtype or *Evf2TS/TS* (Bond et al., 2009) E13.5 ganglionic eminences were diluted to the

same buffer composition as the wash buffer and pre-cleared through the BSA column, then loaded on the anti-*dll* column. Flow-through was reapplied twice to the anti-*dll* column. The column was washed twice, and bound proteins were eluted with 1 ml 0.2 M glycine, pH 2.5 into 70 μ l Tris, pH 9.5. Protein was TCA-precipitated overnight at 4°C, pelleted by centrifugation, and washed with acetone.

Mass spectrometry

Digestion: To each of the samples 60 μ L of 8 M Urea, 100 mM Tris, pH 8.5 were added to solubilize the protein. The subsequent mixture was then reduced by adding 0.3 μ l of 1M TCEP (for a final concentration of 5 mM TCEP) and incubated at room temperature. To alkylate, 1.2 μ l of Iodoacetamide (10 mM final concentration) was added and the samples were subsequently incubated at room temperature while in the dark for 15 minutes. The addition of 180 μ l of 100 mM Tris pH 8.5 diluted the solutions to 2 M Urea. Calcium chloride (100 mM) was then added (2.4 μ l) for a final concentration of 1 mM CaCl₂. Trypsin (0.5 μ g/ μ l) was added in the amount of 7.0 μ l. The resulting mixtures were then shaken for 18 hours and incubated in the dark at 37 °C. To neutralize 13.5 μ l of Formic Acid (90%) was added for a final concentration of 5% Formic Acid. The tubes were centrifuged for 30 minutes at 2 °C on a table top centrifuge.

Multidimensional Protein Identification Technology (MudPIT): Upon completion of the digestion, the proteins were pressure-loaded onto a fused silica capillary desalting column containing 3 cm of 5 μ m strong cation exchange (SCX) followed by 3 cm of 5 μ m C18 (reverse phase or RP material) packed into a

undeactivated 250- μ m i.d capillary. Using 1.5 mL of buffer A (95% water, 5% acetonitrile, and 0.1% formic acid) the desalting columns were washed overnight. Following the desalting process, a 100 μ m i.d capillary consisting of a 10 μ m laser pulled tip packed with 10 cm 3- μ m Aqua C18 material (Phenomenex, Ventura, CA) was attached to the filter union (desalting column– filter union– analytical column) and the entire split-column (desalting column–filter union– analytical column) was placed in line with an Agilent 1100 quaternary HPLC (Palo Alto, CA) and analyzed using a modified 6-step separation described previously (Washburn et al., 2001). The buffer solutions used were 5% acetonitrile/0.1% formic acid (buffer A), 80% acetonitrile/0.1% formic acid (buffer B), and 500 mM ammonium acetate/5% acetonitrile/0.1% formic acid (buffer C). Step 1 consisted of a 90 min gradient from 0-100% buffer B. Steps 2-5 had the following profile: 3 min of 100% buffer A, 2 min of X% buffer C, a 10 min gradient from 0-15% buffer B, and a 97 min gradient from 15-45% buffer B. The 2 min buffer C percentages (X) were 20, 40, 60, 80% respectively for the 6-step analysis. The final step, the gradient contained: 3 min of 100% buffer A, 20 min of 100% buffer C, a 10 min gradient from 0-15% buffer B, and a 107 min gradient from 15-70% buffer B. As peptides eluted from the microcapillary column, they were electrosprayed directly into an LTQ 2-dimensional ion trap mass spectrometer (ThermoFinnigan, Palo Alto, CA) with the application of a distal 2.4 kV spray voltage. A cycle of one full-scan mass spectrum (400-1400 m/z) followed by 8 data-dependent MS/MS spectra at a 35% normalized collision energy was repeated continuously throughout each step of the multidimensional

separation. Application of mass spectrometer scan functions and HPLC solvent gradients were controlled by the Xcalibur datasystem.

Analysis of Tandem Mass Spectra: As each step was executed, its spectra were recorded to a RAW file. This data was then converted into .ms2 format through the use of RawXtract (Version 1.9). From the .ms2 files, poor quality spectra were removed from the dataset using an automated spectral quality assessment algorithm(Bern et al., 2004). MS/MS spectra remaining after filtering were searched with the SEQUEST™ algorithm (Eng et al., 1994) against the NCBI RefSeq Human (04-23-2010) protein database concatenated to a decoy database in which the sequence for each entry in the original database was reversed(Peng et al., 2003). All searches were parallelized and performed on a Beowulf computer cluster consisting of 100 1.2 GHz Athlon CPUs (Sadygov et al., 2002). No enzyme specificity was considered for any search. SEQUEST results were assembled and filtered using the DTASelect (version 2.0) program (Cociorva et al., 2007). DTASelect 2.0 uses a linear discriminant analysis to dynamically set XCorr and DeltaCN thresholds for the entire dataset to achieve a user-specified false positive rate (1% in this analysis). The false positive rates are estimated by the program from the number and quality of spectral matches to the decoy database.

NIR probe transcription

The method for *in vitro* transcription of the NIR probe has been described previously (Kohn et al., 2010). The template for transcription of the probe was generated by PCR amplification of a 115-nucleotide region of wildtype *Evf2* (F

primer: 5'-CCTTTCCTGTTTCCTGAATCTA AA-3', R primer: 5'-TTTTTCATGTAGCCCGCTGAT-3'), and TA-cloning into the pGEM-T Easy Vector (Promega). 1 µg of Sall linearized DNA template, 5 mM DTT, 0.6 µl RNasin (Promega), 0.5 mM ATP, 0.5 mM CTP, 0.5 mM GTP, 12.5 µM UTP, 20 mM Aminoallyl-UTP-Atto680 (Jena Bioscience), 1 µg BSA, and 2 µl (100 U) T7 RNA polymerase were incubated in 20 µl 1X RNA polymerase buffer for 1 hour at 37°C. 2 µl Turbo DNase (Life Technologies) and 2ul 10X Turbo DNase Buffer were added to the reaction and incubated at 37°C for 15 min. The RNA was denatured and separated on a 6% urea-polyacrylamide gel, cast on a Hoeffer miniVE apparatus and pre-run 20 minutes before loading. Full-length probe was excised, eluted overnight at 4°C in 0.5M Ammonium Acetate/1mM EDTA and ethanol precipitated. The concentration of the NIR labeled RNA probe was measured by absorption at 260 nm using the NanoDrop 1000 (Thermo Scientific).

In vitro transcription of RNA competitors

The RNA competitors used in remodeling and ATPase assays (*Evf2* 622 bp, *pGEM* 535 bp, *Dlx1UR* 534 bp and *28S* 630 bp) were generated by in vitro transcription using the following template DNAs: pcDNA-r*Evf2* 3'del5 (Feng et al., 2006) linearized with NotI, pGEM-T Easy Vector (Promega) linearized with PciI, pcDNA-Dlx1UR linearized with XhoI. A schematic for RNAs is included in Table S4. The plasmid for transcription of 28S RNA was generated by PCR amplification

(Primer F: 5'-ACGGACCAAGGAGTCTAACGCGT-3',

Primer R: 5'-CTTAACCCGGCGTTCGGTTCAT-3')

using mouse cDNA as template and TA-cloning into pGEM-T Easy Vector (Promega). pGEM-28S was linearized with SpeI for in vitro transcription.

The linearized templates were treated with Proteinase K (Roche) and ethanol-precipitated. RNA was transcribed as follows: 1.25 µg DNA template, 10 mM DTT, 1.5 µl (80 U) RNasin (Promega), 2 mM A, C, G, and UTP (Roche), and 2 µl (100 U) T7 RNA polymerase (NEB) in 50 µl 1X RNA polymerase buffer were incubated at 37°C for 1 hour. Samples were incubated with 2µl Turbo DNase (Life Technologies) in 1X Turbo DNase Buffer for 15 minutes at 37°C. RNA was treated with Proteinase K (Roche), ethanol-precipitated and quantified using the Quantifluor RNA System (Promega).

PolyU-agarose binding assay

40 mg of Poly U Agarose beads (Sigma) were reconstituted in 1X PBS (200 µl of packed beads). The beads were washed three times with 1mL 1X PBS. 8 picomoles of Brg1 were incubated with ribohomopolymers (2.25 µg, 9 µg, 36 µg) for 15 minutes at room temperature with rotation in 600 µl 20 mM Hepes-KOH pH 7.9, 50 mM KCl, 0.2 mM EDTA, 1 mM DTT containing RNasin (Promega). 10 µl of packed beads were added to each sample and incubated overnight at 4°C. The beads were washed three times with 1mL 20 mM Hepes-KOH pH 7.9, 50 mM KCl, 0.2 mM EDTA and once with 1X PBS. 20 µl of 1X SDS Sample Buffer

was added to the beads for elution. Ribohomopolymers: Poly A (Sigma), Poly G (Sigma), Poly C (Midland), Poly U (Midland)

Fluorescent In Situ Hybridization / Immunohistochemistry Co-labeling

The *Evf2* antisense probe construct was cloned from the 5' 595 bp fragment of rat *Evf2* as previously described (Feng et al., 2006). All steps were performed at room temperature unless stated otherwise. E13.5 embryos were fixed in 4% paraformaldehyde at 4° overnight and then sunk consecutively in 15% and then 30% sucrose in PBS at 4° overnight. Embryos were cryostat sectioned at 18 μm , mounted on slides, and air-dried before staining. Sections were fixed in 4% paraformaldehyde for 10 minutes, washed three times with PBS, then digested with 1 $\mu\text{g}/\text{mL}$ Proteinase K (Roche) in 50 mM Tris pH 7.5 and 5 mM EDTA for 5 minutes. Sections were refixed in 4% paraformaldehyde and washed three times with PBS. Slides were then incubated for 10 minutes in acetylation solution (40 mL ddH₂O, 0.73 g triethanolamine, 90 μl 10 N NaOH) to which 100 μl of acetic anhydride was added dropwise with agitation. After three washes in PBS, sections were incubated in 1% Triton-X in PBS for 1 hour. Endogenous peroxidase activity was quenched by incubation in 1% hydrogen peroxide in PBS for 30 minutes, followed by three washes with PBS. 300 μl of prehybridization buffer was added to each slide and sections were incubated under parafilm overnight in a humidified chamber. Probe hybridization was performed by adding 150 μl of Digoxigenin-labeled *Evf2* antisense probe diluted in prehybridization buffer to each slide and incubating overnight at 72° in a humidified chamber.

Slides were washed with 5x SSC, incubated in 0.2x SSC for 1 hour at 72°, washed with 0.2x SSC, washed three times with PBS and incubated in 0.5% Triton-X in PBS for 3 minutes. Sections were blocked with 1% TSA blocking reagent (TSA kit #2, Invitrogen), then incubated overnight in a humidified chamber at 4° overnight with mouse monoclonal anti-Digoxigenin (11 333 062 910, Roche) and rabbit polyclonal anti-BRG1 (Wang et al., 1996) diluted 1:500 and 1:400 respectively in 1% TSA blocking reagent. After three washes with PBS, slides were incubated with HRP-goat anti-mouse IgG for 1 hour and tyramide labeled with Alexa Fluor 488 as directed by manufacturer's instructions (TSA kit #2). After washing twice with PBS, slides were incubated in 1% hydrogen peroxide for 30 minutes to quench residual HRP activity, then reblocked in 1% TSA blocking reagent for 1 hour. For second round tyramide labeling, slides were incubated with HRP-goat anti-rabbit HRP and labeled with Alexa Fluor 568 (TSA kit #14, Invitrogen). Slides were washed three times in PBS, then stained with DAPI for 5 minutes before mounting in FluorSave reagent (Calbiochem). A Zeiss 510 Meta Confocal microscope with ZEN software was used to visualize fluorescence. All chemicals were obtained from Sigma-Aldrich unless otherwise specified.

ChIP-qPCR

Whole ganglionic eminences were dissected from 10 wild type and 10 *Evf2* TS/TS E13.5 embryos. Tissues were pooled for each genotype, triturated by pipetting, and filtered through a cell-strainer capped 5 ml polystyrene round-

bottom tube (BD Falcon) to make single-cell suspensions. Samples were fixed in 1% paraformaldehyde for 90 min (10 min for Brg1 ChIP), then lysed in SDS lysis buffer (1% SDS, 50 mM Tris-HCl pH 8, 10 mM EDTA) with protease inhibitors (11836153001, Roche). The lysates were sonicated with a Microson Ultrasonic Cell Disruptor using 6 pulses of 10 seconds at 9 watts (RMS). The lysates were then centrifuged to pellet cellular debris and the supernatant collected for ChIP.

Chromatin Immunoprecipitation

Rabbit pan-antibodies to DLX were generated in our laboratory and previously characterized (Feng et al., 2006). Rabbit polyclonal anti-BRG1 was previously described (Wang et al., 1996). Rabbit polyclonal anti-Histone H3 (9715) was obtained from Cell Signaling Technologies. The following rabbit polyclonal antibodies were purchased from Abcam: acetylated H3K9 (ab10812), acetylated H3K18 (ab1191), and acetylated H3K27 (ab4729), and following antibodies were purchased from Active Motif: H4AcK (39926), H4K5Ac (39700).

25 μ g of chromatin were diluted 1:10 in RIPA Buffer (10mM Tris pH 7.6, 1mM EDTA, 0.1% SDS, 0.1% Sodium Deoxycholate, 1% Triton X-100) with protease inhibitors (11836153001, Roche). The chromatin was pre-cleared by rotating at 4°C with 50 μ l of Protein G–Agarose beads (11719416001, Roche) for 1 hour. After centrifugation to pellet the beads, the supernatant was further pre-cleared by rotating at 4°C with 50 μ l rabbit IgG conjugated Protein G–Agarose beads for 1 hour. The pre-cleared chromatin was incubated with rabbit IgG (2 μ g or 5 μ g), or antibodies targeting pan-DLX (5 μ g), BRG1 (5 μ g), and 2 μ g targeting

the following: Histone H3, H3K9ac, H3K18ac, H3K27ac, H4acK, H4K5ac, at 4°C for 4 hours with rotation. 50 μ l of Protein G-Agarose beads blocked with 1% BSA in 1X PBS were added to each sample and incubated at 4°C overnight with rotation. Beads were pelleted by centrifugation and washed twice with Low Salt Buffer (20 mM Tris-HCl pH 8.1, 2 mM EDTA, 150 mM NaCl, 0.1% SDS, 1% Triton X-100), three times with High Salt Buffer (20 mM Tris-HCl pH 8.1, 2 mM EDTA, 500 mM NaCl, 0.1% SDS, 1% Triton X-100), four times with LiCl buffer (0.25M LiCl, 10 mM Tris-HCl pH 8.1, 1 mM EDTA, 1% sodium deoxycholate and 1% NP-40), twice with 0.1% Tween-20 in 1X PBS, and once with TE buffer (10 mM Tris-HCl pH 8.1 and 1 mM EDTA). Immunoprecipitated DNA was eluted from the beads by incubation with 200 μ l of elution buffer (50 mM Tris-HCl pH 8, 10 mM EDTA, 1% SDS) at 65°C for 1 hour. The beads were removed by centrifugation and DNA crosslinking was reversed at 65°C for 4 hours. The Immunoprecipitated DNA was purified using the Qiaquick PCR Purification Kit (Qiagen).

ChIP qPCR

Immunoprecipitated DNA diluted 1:20 in ddH₂O, primers, and Perfecta SYBR Green FastMix (Quanta Biosciences) were combined to make 20 μ l reactions in a MicroAmp Fast Optical 96-well Reaction Plate (Life Technologies). Quantification of the immunoprecipitated material was performed in the Fast 7500 Real-Time PCR System (Life Technologies). The percent input of each antibody was determined by the following formula: $2^{-(Ct(\text{antibody})-Ct(\text{Input}))} - 2^{-(Ct(\text{IgG})-Ct(\text{Input}))}$

$C(\text{Input})$. Statistics were obtained using an unpaired student's *t*-test with equal variance.

Anti-DLX ChIP, reChIP

The ChIP protocol described above was used to perform anti-Dlx ChIP, followed by elution and re-immunoprecipitation with anti-BRG1, anti-lamin B1 or anti-IgG, as described in (Truax and Greer, 2012). %Input values obtained for IgG were subtracted from anti-BRG1 and anti-lamin B1 values. Statistics were obtained using an unpaired student's *t*-test with equal variance.

BRG1 ATPase and remodeling assays

Protein purification and nucleosome assembly: Flag-epitope Brg1 and Brg1 mutants were purified from Sf9 cells using a baculovirus expression as previously described (Phelan et al, 1999). Recombinant histones were purified and reconstituted into octamer as described previously (Luger et al, 1999). Mononucleosomes were assembled onto a 237 nucleotide DNA fragment containing a central 601 positioning sequence that had been modified to contain an internal PstI cleavage site.

ATPase assays: Reactions were performed in 8 mM HEPES (pH 7.9), 10 mM Tris (pH 7.5), 60 mM KCl, 3 mM MgCl₂, 8% glycerol in 10 mL at 30°C. Various concentrations of the different RNAs were incubated with BRG1 for 30 minutes prior to initiating the reaction by adding α -³²P-ATP to the mix. Reactions proceeded for one hour and then were stopped by adding 10 mL of 200 mM EDTA. ATP and ADP were separated by PEI-cellulose TLC using 0.75 M

KH₂PO₄. Extent of inhibition was quantified using a Typhoon scanner and ImageQuant software.

Restriction Enzyme Accessibility: Reactions were carried out as described above, in the presence of 0.1 U/mL PstI and initiated by the addition of labeled nucleosomes. Reactions were stopped by the addition of 15-ml of stop buffer (1.5 mg/ml Proteinase K, 70 mM EDTA, 10 mM Tris (pH 7.7), 1% SDS, 0.1% Orange-G). Reactions were incubated at 55 °C for 1 hour and then separated on an 8 % acrylamide gel in 1 X TBE. DNA was visualized on a Typhoon scanner and quantified using ImageQuant software. Apparent inhibition of remodeling was determined by the equation:

$$\frac{(\text{fraction uncut with RNA and Brg1} - \text{fraction uncut with Brg1})}{(\text{fraction uncut without Brg1} - \text{fraction uncut with Brg1})}$$

and plotted using Graphpad Prism and fit to an equation for a sigmoidal curve.

Primer Information:

The following primers were used in ChIP experiments:

Dlx5/6 1-F (1.2 μM, 5'-CTTCTTCCTACCTGAAGCAG-3'), Dlx5/6 1-R (1.2 μM, 5'-ACACTTCCAAGTGTGAAGCC-3'),
Dlx5/6 2-F (0.7 μM, 5'-CCCAGGATCAATTCTGAACAAAG-3'), Dlx5/6 2-R (0.7 μM, 5'-TCCCAATGTCTGCTTCAAAT-3'),
Dlx5/6 3-F (1 μM, 5'-GGCGCATCTTTGCAAATTACA-3'), Dlx5/6 3-R (1 μM, 5'-GCAGGCTGGATTAGGATGCTA-3'),
Dlx5/6 4-F (1.2 μM, 5'-TCGAAAGTATTGCGTGGATG-3'), Dlx5/6 4-R (1.2 μM, 5'-GTGTGTACCAAGCGCATGTC-3'),
Dlx1 UR-F (0.7 μM, 5'-AAAAGGCCCTGGATGCTCTT-3'), Dlx1 UR-R (0.7 μM, 5'-GAGCAAGCGTGCAGATGAGA-3'),
Ptc1 UR-F (1 μM, 5'-GAACCGCACTAGGTACCCAT-3'), Ptc1 UR-R (1 μM, 5'-CATGGGTGTGAGGACACGTT-3'),

Shh UR-F (1 μ M, 5'-GACCCCATGGAGCAGGTTT-3'), Shh UR-R (1 μ M, 5'-GTCCCTTGTCCCTGCGTTTCA-3'),

Sequences of RNAs used in ATPase and remodeling assays and REMSAs
(schematics in Table S4):

Blue regions are either pGEM or pcDNA

pcDNA-Evf2-(5' end) length: 622

GGGAGACCCAAGCTTGGTACCGAGCTCGGATCGATCCCCCGGGCTGCAGG
AATTCGGCACGAGGACAGAGCTTCTGCCCGAGACTCAGAAAAATACTCTC
CTGTGCCTCGGCTCAGTATAGATTTCTAGACCCTGATCATTGCTTAAGAGAG
ATTCTCTGGGGTCCCTCAGTCTCTGCAATTTGTGTATGAATAACAGAATAATTT
CCCTCTTTTGTTCGCCTTTCCTGTTCCCTGAATCTAAATAAAGATGGCTTTTT
AGTATTAAGAGTGAAGAAAATTACAGGTAATTATCTTTGACGGTAAAAACGC
TGTAATCAGCGGGCTACATGAAAAATTACTCTAATTATGGCTGCATTTAAGAG
AATGGAAAAAACCTTCTTGTGGATAAAAAACCTTAAATTGTCCCAATGTCTG
CTTCAAATTGGATGGCACTGCAGCTGGAGGCTTTGTTTCAAGATTGATCCTGG
GGAGCTACAAACCCAAAGTTTCACAGTAGGAAGGGGGAAAAAAGAAAAGAA
AACATTTTTCCTAATGTAACAATGCGAATGGTAGAAAATGACAAGACTGATCG
GTTTTAAACCATTCTGAAGACTGACTGAGTGTGATCCATCACACTGGC

pcDNA-Dlx1UR length: 606

GGGAGACCCAAGCTTGGTACCGAGCTCGGATCCACTAGTAACGGCCGCCA
GTGTGCTGGAATTCTGCAGATATCCATCACACTGGCGGCCGCGGGAATTCCG
ATTGCTTTTCAAGGACTAGCTGGAGTTCACCTGCATGTTGGTGTGGCGTGTG
GCTCTTTTTTGTAAAGAGTTAAGTCGTATACAAAAGGGGGAAACAAAGGTCA
GCGCCAGCCACCACTTGAATGTGAGATTTTTCTTTTTATTCCCCTTGATGTA
TACTAGGCTCTGTGTTATTAGTCTTAATGATGGAAAATTGTAGGGTTGATACA
AATCTTTTTTCAAAGTAGTAGGCGGGAGCTCCATTTGTTAGTAAGTTTTTTT
GTGGCTAAAAGCCACGGACAGTACATTTATGTAGCAGTAAAAGGCCCTGGAT
GCTCTTTCCATAGCAGAGCTAATTATTCCTACTGTGACCTTACTGATCTAGCC
TGTTCCAGCACATCTCATCTGCACGCTTGCTCCTCTGTGTAGATGGTGTCA
GGGAATATGAAAAACCCTATAATGAAACCATTTTCATGATGGAGAAAGGCTA
CTGGAGTCGT**ATCACTAGTGAATTCGCGGCCGC**

pGEM-28S length: 638

GGGCGAATTGGGCCCGACGTCGCATGCTCCCGGCCGCCATGGCGGCCGCG
GGAATTCGATTACGGACCAAGGAGTCTAACGCGTGCAGGAGTCAGGGGCTC
GTCCGAAAGCCGCCGTGGCGCAATGAAGGTGAAGGGCCCCGCCCGGGG
CCCGAGGTGGGATCCCGAGGCCTCTCCAGTCCGCCGAGGGCGCACACCAG
GCCCGTCTCGCCCGCCGCGCGGGGAGGTGGAGCACGAACGTACGCGTTA
GGACCCGAAAGATGGTGAACCTATGCCTGGGCAGGGCGAAGCCAGAGGAAA
CTCTGGTGGAGGTCCGTAGCGGTCCTGACGTGCAAATCGGTCTCCGACCT
GGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTTCCCTCCG

AAGTTTCCCTCAGGATAGCTGGCGCTCTCGCTCCCGACGTACGCAGTTTTAT
CCGGTAAAGCGAATGATTAGAGGTCTTGGGGCCGAAACGATCTCAACCTATT
CTCAAACCTTTAAATGGGTAAGAAGCCCGGCTCGCTGGCGTGGAGCCGGGCG
TGGAATGCGAGTGCCTAGTGGGCCACTTTTGGTAAGCAGAACTGGCGCTGC
GGGATGAACCGAACGCCGGGTTAAGAATCA

pGEM-T Easy length: 517

GGGCGAATTGGGCCCACGTCGCATGCTCCCGGCCGCCATGGCGGCCGCG
GGAATTCGATATCACTAGTGAATTCGCGGCCGCCTGCAGGTGCACCATATG
GGAGAGCTCCCAACGCGTTGGATGCATAGCTTGAGTATTCTATAGTGTCCACC
TAAATAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTAT
CCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAGCCT
GGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCC
CGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCA
ACGCGCGGGGAGAGGGCGGTTTGCATTGGGCGCTCTTCCGCTTCCCTCGCT
CACTGACTCGCTGCGCTCGGTCGTTCCGGCTGCGGCGAGCGGTATCAGCTCA
CTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAA
GAA

RNAs used in REMSAs:

pGEM-Evf2-(UCR) length: 206

GGGCGAATTGGGCCCACGTCGCATGCTCCCGGCCGCCATGGCGGCCGCG
GGAATTCGATTCCTTTCTGTTCCCTGAATCTAAATAAAGATGGCTTTTTAGTA
TTAAAAGTGGAAGAAAATTACAGGTAATTATCTTTGACGGTAAAAACGCTGTA
ATCAGCGGGCTACATGAAAAATCACTAGTGAATTCGCGGCCGCCTGCAGG

pGEM-Dlx1UR length: 180

GGGCGAATTGGGCCCACGTCGCATGCTCCCGGCCGCCATGGCGGCCGCG
GGAATTCGATTTGTAAAGAGTTAAGTCGTATACAAAAGGGGGAACAAAGGT
CAGCGCCAGCCACCACTTGAATGTGAGATTTTTCTTTTTATTCCCCTTTCAC
TAGTGAATTCGCGGCCGCCTGCAGG

pGEM-28S length: 160

GGGCGAATTGGGCCCACGTCGCATGCTCCCGGCCGCCATGGCGGCCGCG
GGAATTCGATTACGGACCAAGGAGTCTAACGCGTGCGCGAGTCAGGGGCTC
GTCCGAAAGCCGCGGTGGCGCAATGAAGGTGAAGGGCCCCGCCCCGGGGG
CCCGAGGTGG

pGEM-T Easy length: 209

GGGCGAATTGGGCCCACGTCGCATGCTCCCGGCCGCCATGGCGGCCGCG
GGAATTCGATATCACTAGTGAATTCGCGGCCGCCTGCAGGTGCACCATATG
GGAGAGCTCCCAACGCGTTGGATGCATAGCTTGAGTATTCTATAGTGTCCACC

TAAATAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTAT
CCG

Data analysis

Schematic for BRG1 domains, with known interactive domains was generated using SMART 7 (Letunic et al., 2012).

RNA binding prediction was performed using BINDN+(Wang and Brown, 2006).

Ultraconserved RNA sequences

CR numbers correspond to Woolfe et al. 2005 database. Primer set numbers correspond to Supplementary Table S3. Yellow highlights indicate 5' and 3' primer sites.

Dlx1UR 479 bp (UCR#10)

CR846130 5' B: TGCTTTGCAAGGACTAGCTG

CR846130 3' A: ACGACTCCAGTAGCCTTTCT

TGCTTTGCAAGGACTAGCTG GAGTTCACCTGCATGTTGGTGTGGCGTGTGG
CTCTTTTTTGTAAAGAGTTAAGTCGTATACAAAAAGGGGGAACAAAGGTCAG
CGCCCAGCCACCACTTGAATGTGAGATTTTTCTTTTTATTCCCCTTGATGTAT
ACTAGGCTCTGTGTTATTAGTCTTAATGATGGAAAATTGTAGGGTTGATACAA
ATCTTTTTTCAAAGTAGTAGGCGGGAGCTCCATTTGTTAGTAAGTTTTTTTG
TGGCTAAAAGCCACGGACAGTACATTTATGTAGCAGTAAAAGGCCCTGGATG
CTCTTTCCATAGCAGAGCTAATTATTCCTACTGTGACCTTACTGATCTAGCCT
GTTCCCAGCACATCTCATCTGCACGCTTGCTCCTCTGTGTAGATGGTGTGTCAG
GGAATATGAAAAACCCTATAATGAAACCATTTTCATGATGG **AGAAAGGCTAC**
TGGAGTCGT

Ptc1UR 411bp (UCR primer set #24)

CR846217 5'A: ATCTGAGAACCGCACTAGGT

CR846217 3'A: ACATTTACATTAAGGACTCATGGTAGGT

ATCTGAGAACCGCACTAGGT CACCATGTTACACCGATACTTTCCAATCACA
TGGATCACTGCTCACGTGTACTCTCCTAACACTGTTAAAACATACTCTCTTCT
CTCCCTTGCTGTCATTTATAACGAAACGTGTCCTCACACCCATGTCTCAGCA
AAGTTCCAGACATTATGGATTTTCATCACATATAAATTCTTTAAAAATATACTTC

TGTCGAAAGACTTGATGACTACTATGTACACTACAAAAATAAATTTTCATATA
AATAAATTATATGGCATACTTTTCATCTTTGTAATTGAAGTGACACAAACTCATT
TCCACCCAAATTGGCAGACGAGACCCAGTTTGAATATTTATTTCCCTTTAAACT
CCTTACACTAAAACCTACCATGAGTCCTTAATGTAAATGT

ShhUR 310bp (UCR primer set #33)

CR847491 5'B: TCTACATGTCCCTTGTCCCTGCGTT

CR847491 3' A: CCAAGAACATGACAAAGTGGCGGT

CCAAGAACATGACAAAGTGGCGGT TACAAAGCAAATACAAACCAAGAAGGC
AGTACAGAAGACGGCTATTACATTGGAAAATAGTTAAATTTAAAATAATATATT
TTCATATTTTACACTTTCAAAAAATGAAATGTCATTCAGTTAGGTATTGATCTC
TCAAAAATCTAATTTACAATTCTGTGTATAAAAATATAATTTGTGGACCCCAT
GGAGCAGGTTTTAGTTTACACTTGCGGGAAAGCAGGAGCATAGCAGGAGAG
GAATGCGGAGGTTTGCCTTCTGA AACGCAGGACAAGGGACATGTAGA

Supplementary References

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Evf2+/+: Total 87				
Evf2+/+-specific proteins in one of the categories listed: 32				
Identifier	seq coverage	seq count	spec count	description
Chromatin remodelers: 8				
IPI00381019. 3.6;3.5;3.3;		2	2	Splice Isoform 2 of SMARCC2 (BAF170, SWI)
IPI00460668. 1.9;2.5;		2	2	SMARCA4 (BRG1, SWI)
IPI00396739. 2.3;2.4;2.5;6.3;		2	2	SMARCA5 (SNF2H, ISWI)
IPI00314654. 2.5;		2	2	SNF2L (ISWI)
IPI00130597. 1.6;		2	2	Bromodomain adjacent to zinc finger domain protein 1B ;
IPI00461396. 1.7;1.9;		2	2	PREDICTED: bromodomain adjacent to zinc finger domain 1A
IPI00648459. 1.5;1.6;		2	2	PREDICTED: AT rich interactive domain 1A, ARID1A, isoform 3 and 2
IPI00461676. 2.6;2.7;2.8;2.6;2.8;2		3	3	PREDICTED: polybromo 1 isoform 1
Chromatin: 2				
IPI00342766. 3.4;2.2;3.7;3.5;		2	2	Heterochromatin protein 1, binding protein 3
IPI00117074. 2.7;2.6;		2	2	Structural maintenance of chromosome 2-like 1 protein
Transcription factors: 6				
IPI00229721. 8.8;		3	3	Polymerase delta-interacting protein 3 ;
IPI00322492. 7.1;7.2;7.6;		3	6	Ewing sarcoma homolog
IPI00169477. 2.6;2.6;		2	2	Splice Isoform 2 of Bcl-2-associated transcription factor 1
IPI00556768. 2.5;		2	2	Thyroid hormone receptor-associated protein 3 ;
IPI00380766. 2.4;2.8;		2	2	Splice Isoform 1 of Ubiquitin-protein ligase BRE1A
IPI00120344. 2.1;		2	3	Chromatin-specific transcription elongation factor, 140 kDa subunit ;
RNA Helicases: 7				
IPI00120691. 4.7;4.7;		3	3	Nucleolar RNA helicase 2 ; DEAD (Asp-Glu-Ala-Asp) box polypeptide 21 ;
IPI00396797. 3.8;3.8;		2	2	Splice Isoform 1 of Probable ATP-dependent RNA helicase DDX17
IPI00339468. 3;3;3.5;		2	2	Splice Isoform 1 of ATP-dependent RNA helicase A;Splice Isoform 2; Splice Isoform 3
IPI00127172. 2.7;		2	2	ATP-dependent RNA helicase DDX1 ;
IPI00120691. 4.7;4.7;		3	3	Nucleolar RNA helicase 2 ; DEAD (Asp-Glu-Ala-Asp) box polypeptide 21 ;
IPI00420363. 3.3;3.3;4.9;		2	2	Probable ATP-dependent RNA helicase DDX5 ; 69 kDa protein ; 46 kDa protein
IPI00133708. 3.3;3.3;3.3;		2	2	Putative ATP-dependent RNA helicase P110 ; DDX3X ; DDX3Y ;
Splicing: 3				
IPI00129430. 12;11.4;10.7;		7	9	Splicing factor, proline- and glutamine-rich ; PREDICTED: NonO/p54nrb homolog;isoform 3
IPI00420807. 7.3;		3	5	Splicing factor, arginine/serine-rich 1 ;
IPI00310880. 6.2;		2	2	Splicing factor, arginine/serine-rich 6,
Polyadenylation: 1				
IPI00110363. 1.9;2.1;1.9;		2	2	Cleavage and polyadenylation specificity factor, 160 kDa subunit
HnRNPs: 5				
IPI00269661. 11.9;15.2;14.8;14.8;		3	6	Splice Isoform 1 of hnRNP A3
IPI00124979. 6.4;6.4;6.4;		2	2	hnRNP G
IPI00136702. 3.5;3.9;3.5;		3	4	NS1-associated protein 1 isoform 1 ; Splice Isoform 2 of HnRNP Q, Isoform 1
IPI00553777. 26;26.1;		6	7	hnRNP A1
IPI00756515. 6;		4	4	PREDICTED: similar to hnRNP U ;

Evf2TS/TS total: 15				
proteins found in Evf2TS/TS and Evf2+/+: 8				
Identifier	seq coverage	seq count	spec count	description
Chromatin association: 2				
IPI00109764.1;2.7;		2	2	DNA topoisomerase 1 ;
IPI00135443.2;1.7;		2	2	DNA topoisomerase 2-beta ;
Transcription factors: 3				
IPI00133259.1;15.7;15.7;		3	4	Homeobox protein DLX-1 ;IPI00349626.2 Tax_Id=10090 27 kDa protein ;
IPI00108386.1;9.3;		2	3	Splice Isoform 1 of Homeobox protein DLX-5 ;
IPI00320016.7;6.3;		2	2	Splice Isoform 1 of Non-POU domain-containing octamer-binding protein ;
RNA Helicases:1				
IPI00420363.2;4.1;4.1;		2	3	Probable ATP-dependent RNA helicase DDX5
HnRNPs:				
IPI00315227.5;5.9;5.7;6.6;		2	2	hnRNP A2/B1
IPI00458583.2;4.9;5.8;		3	7	hnRNP U,
Other:				
IPI00311103.1;17.9;17.9;		4	4	Splice Isoform 1 of RNA and export factor-binding protein 2
IPI00135686.2;12;		2	2	peptidylprolyl isomerase B ;
IPI00753135.1;10.9;		3	4	Splice Isoform Epsilon of Lamina-associated polypeptide 2 isoforms beta/delta/epsilon/gamma ;
IPI00223714.4;10.6;		2	2	Histone H1.4 ;
IPI00331628.4;4.9;		3	3	Peroxisomal multifunctional enzyme type 2 ;
IPI00130920.1;1.1;		2	2	Microtubule-associated protein 1B ;
IPI00553419.3;0.7;0.8;0.7;0.9;		2	2	PREDICTED: desmoplakin isoform 1
Chromatin remodelers (SWI, ISWI, BROMO): 0				
Splicing: 0				
Polyadenylation: 0				

Complete list of proteins identified in the DLX bound complex in *Evf2^{+/-}* nuclear extracts

locus	seq coverage	seq count	spec count	description
IP00331597.27.3;	8	16	16	IP00331597.27.3; ;
IP0055377.26.26.1;	6	7	7	IP0055377.26.26.1; ;
IP00119220.24.6.24.3;	3	3	3	IP00119220.24.6.24.3; ;
IP00315227.22.9.22.1,25;	6	7	7	IP00315227.22.9.22.1,25; ;
IP00118590.20.7;	4	4	4	IP00118590.20.7; ;
IP0031612.20.4.17.1;	2	3	3	IP0031612.20.4.17.1; ;
IP00223714.20.2;	7	14	14	IP00223714.20.2; ;
IP00228616.14.6;	3	4	4	IP00228616.14.6; ;
IP00268802.13.2.13.2,13;	2	2	2	IP00268802.13.2.13.2,13; ;
IP00129430.12.11.4.10,7;	7	7	7	IP00129430.12.11.4.10,7; ;
IP00269611.14.11.6.15.2,14;	3	3	3	IP00269611.14.11.6.15.2,14; ;
IP00108454.10.10.10.8,10,10;	2	2	2	IP00108454.10.10.10.8,10,10; ;
IP00134599.20.7;	2	4	4	IP00134599.20.7; ;
IP00227299.10.5;	4	4	4	IP00227299.10.5; ;
IP00230133.10.4;	4	5	5	IP00230133.10.4; ;
IP00453865.9.3;	2	2	2	IP00453865.9.3; ;
IP00622371.9;	2	2	2	IP00622371.9; ;
IP00229721.8.8;	3	3	3	IP00229721.8.8; ;
IP00318841.14.10.6.8,8,7,10;	2	2	2	IP00318841.14.10.6.8,8,7,10; ;
IP00133259.7.8,7,8;	2	2	2	IP00133259.7.8,7,8; ;
IP00420726.7.8;	2	4	4	IP00420726.7.8; ;
IP00458583.7.4,8,8,8,7;	6	6	6	IP00458583.7.4,8,8,8,7; ;
IP00311236.7.4,8,11,7,4;	4	4	4	IP00311236.7.4,8,11,7,4; ;
IP00457741.7.3;	4	4	4	IP00457741.7.3; ;
IP00420807.7.3;	3	3	3	IP00420807.7.3; ;
IP00254652.7.2,7,7;	2	2	2	IP00254652.7.2,7,7; ;
IP00133985.1.2;	2	2	2	IP00133985.1.2; ;
IP0032492.7.1,7,2,7,6;	3	6	6	IP0032492.7.1,7,2,7,6; ;
IP00269613.6.8,13,3;	2	2	2	IP00269613.6.8,13,3; ;
IP00308706.6.8,9;	2	2	2	IP00308706.6.8,9; ;
IP00122421.14.7.6.6,8.6,6;	2	2	2	IP00122421.14.7.6.6,8.6,6; ;
IP00130591.6.6,6,7;	3	3	3	IP00130591.6.6,6,7; ;
IP00469268.4.4;	3	3	3	IP00469268.4.4; ;
IP00124979.4.6.4,6,4,6,4;	2	2	2	IP00124979.4.6.4,6,4,6,4; ;
IP00320016.6.3;	2	2	2	IP00320016.6.3; ;
IP00310880.6.2;	2	2	2	IP00310880.6.2; ;
IP00756515.6;	4	4	4	IP00756515.6; ;
IP00467338.6;	2	2	2	IP00467338.6; ;
IP00230394.5.8;	2	2	2	IP00230394.5.8; ;
IP00457756.5.6;	2	2	2	IP00457756.5.6; ;
IP00120691.4.7,4,7;	3	3	3	IP00120691.4.7,4,7; ;
IP00223047.4.7;	2	2	2	IP00223047.4.7; ;
IP00169793.4.6,6,6,6,6,6;	2	2	2	IP00169793.4.6,6,6,6,6,6; ;
IP00387329.4.5,5;	2	2	2	IP00387329.4.5,5; ;
IP00263048.4.3;	6	6	6	IP00263048.4.3; ;
IP00317794.4.2;	4	4	4	IP00317794.4.2; ;
IP00118678.4.14,14,1;	2	2	2	IP00118678.4.14,14,1; ;
IP00338604.3.8,3,8,3,9;	6	6	6	IP00338604.3.8,3,8,3,9; ;
IP00622235.3.8,3,9;	2	2	2	IP00622235.3.8,3,9; ;
IP0039697.3.8,3,8;	2	2	2	IP0039697.3.8,3,8; ;
IP00381019.3.6,3,5,3,3;	2	2	2	IP00381019.3.6,3,5,3,3; ;
IP00136702.3.5,3,9,3,5;	3	3	3	IP00136702.3.5,3,9,3,5; ;
IP00312128.3.4;	2	2	2	IP00312128.3.4; ;
IP00122521.3.4,3,5,3,5,3;	2	2	2	IP00122521.3.4,3,5,3,5,3; ;
IP00342766.3.4,2,3,7,3;	2	2	2	IP00342766.3.4,2,3,7,3; ;
IP00130920.3.3;	5	5	5	IP00130920.3.3; ;
IP00133708.3.3,3,3,3,3;	2	2	2	IP00133708.3.3,3,3,3,3; ;
IP00420363.3.3,3,4,9;	2	2	2	IP00420363.3.3,3,4,9; ;
IP00108389.3.2,3,1;	4	4	4	IP00108389.3.2,3,1; ;
IP00339468.3.3,3,5;	2	2	2	IP00339468.3.3,3,5; ;
IP00109764.3;	2	2	2	IP00109764.3; ;
IP00117074.7.2,6;	2	2	2	IP00117074.7.2,6; ;
IP00127172.7.2;	2	2	2	IP00127172.7.2; ;
IP00461676.2.6,2,7,2,8,2;	3	3	3	IP00461676.2.6,2,7,2,8,2; ;
IP00169477.1.6,2,6,2;	2	2	2	IP00169477.1.6,2,6,2; ;
IP00117731.5.2,5,2,5,2;	2	2	2	IP00117731.5.2,5,2,5,2; ;
IP00314654.2.5;	2	2	2	IP00314654.2.5; ;
IP00556768.2.5;	2	2	2	IP00556768.2.5; ;
IP00380766.2.4,2,8;	2	2	2	IP00380766.2.4,2,8; ;
IP00754183.2.3;	2	2	2	IP00754183.2.3; ;
IP00169916.2.3,2,3;	3	3	3	IP00169916.2.3,2,3; ;
IP00122223.2.3;	2	2	2	IP00122223.2.3; ;
IP00123870.2.3;	2	2	2	IP00123870.2.3; ;
IP00396739.1.3,2,4,2,5,6;	2	2	2	IP00396739.1.3,2,4,2,5,6; ;
IP00121892.2.2,2;	3	3	3	IP00121892.2.2,2; ;
IP00118075.2.2,2,5,2,3,2;	3	3	3	IP00118075.2.2,2,5,2,3,2; ;
IP00469323.2.4;	2	2	2	IP00469323.2.4; ;
IP00135432.2.2;	2	2	2	IP00135432.2.2; ;
IP00120344.2.1;	2	2	2	IP00120344.2.1; ;
IP00124959.2;	4	4	4	IP00124959.2; ;
IP00460668.1.9,2,5;	2	2	2	IP00460668.1.9,2,5; ;
IP00110361.1.9,2,11,9;	2	2	2	IP00110361.1.9,2,11,9; ;
IP00461396.1.7,1,9;	2	2	2	IP00461396.1.7,1,9; ;
IP00757359.1.7;	2	2	2	IP00757359.1.7; ;
IP00130597.1.6;	2	2	2	IP00130597.1.6; ;
IP00648659.1.5,1,6;	2	2	2	IP00648659.1.5,1,6; ;
IP00464313.1.4;	2	2	2	IP00464313.1.4; ;

UCRs from Woolfe et al 2005 database:

expressed in mouse brain EST database, and as opposite strand (OS) or antisense (AS) transcrip

Successful RT-PCR from E13.5 ganglionic eminence

Subcloned in pGEM, sequence verified

Set #	UCR	5' Primer	3' Primer	Expected Product		Tm of Primers (5'/3')
Set1	Shh/HlxB9	CR847029 A	CR847029 A	280 bp	includes part UCR	55/52
Set2	Shh/HlxB9	CR847029 A	CR847029 B	343 bp	includes part UCR	55/55
Set3	Dlx1/2	CR846134 A	CR846134 A	778 bp	includes whole UCR	60
Set4	Dlx1/2	CR846134 B	CR846134 A	314 bp	includes whole UCR	55/60
Set5	Dlx1/2	CR846134 B	CR846134 B	277 bp	includes whole UCR	55
Set6	Dlx1/2	CR846134 A	CR846134 B	741 bp	includes whole UCR	60/55
Set7	Dlx1/2	CR846130 A	CR846130 A	373 bp	includes part UCR	55
Set8	Dlx1/2	CR846130 B	CR846130 B	394 bp	includes part UCR	55
Set9	Dlx1/2	CR846130 A	CR846130 B	287 bp	includes part UCR	55
Set10	Dlx1/2	CR846130 B	CR846130 A	479 bp	includes part UCR	55
Set11	Dlx1/2	CR846143 A	CR846143 A	200 bp	includes part UCR	56/57
Set12	Dlx1/2	CR846143 A	CR846143 B	420 bp	includes part UCR	57
Set13	Gbx2	CR847118 A	CR847118 A	395 bp	includes part UCR	57/55
Set14	Gbx2	CR847118 A	CR847118 B	290 bp	includes part UCR	57/56
Set15	Gbx2	CR847118 B	CR847118 B	426 bp	includes part UCR	53/56
Set16	Gbx2	CR847118 B	CR847118 A	531 bp	includes part UCR	53/55
Set17	Meis1	CR846655 A	CR846655 A	336 bp	includes whole UCR	60
Set18	Meis1	CR846655 B	CR846655 A	357 bp	includes whole UCR	60
Set19	Nkx6.1	CR847335 A	CR847335 A	221 bp	includes part UCR	57/55
Set20	Nkx6.1	CR847335 A	CR847335 B	181 bp	includes part UCR	57/56
Set21	Nkx6.1	CR847340/38 A	CR847340/38 A	499 bp	includes both UCRs (whole)	55/56
Set22	Nkx6.1	CR847340/38 A	CR847340/38 B	271 bp	includes whole 847338 UCR	55/55
Set23	Nkx6.1	CR847340/38 B	CR847340/38 A	202 bp	includes whole 847340 UCR	55/56
Set24	Ptch	CR846217 A	CR846217 A	411 bp	includes whole UCR	55/56
Set25	Ptch	CR846217 A	CR846217 B	337 bp	includes whole UCR	55/56
Set26	Ptch	CR846217 B	CR846217 A	312 bp	includes whole UCR	56/56
Set27	Ptch	CR846217 B	CR846217 B	238 bp	includes whole UCR	56/56
Set28	Zic1	CR846271 A	CR846271 A	387 bp	includes part UCR	56/56
Set29	Zic1	CR846271 A	CR846271 B	631 bp	includes part UCR	56/55
Set30	Zic1	CR846271 B	CR846271 A	449 bp	includes part UCR	57/56
Set31	Zic1	CR846271 B	CR846271 B	694 bp	includes part UCR	57/55
Set32	Shh/HlxB9	CR847491 A	CR847491 A	585 bp	includes whole UCR	60
Set33	Shh/HlxB9	CR847491 B	CR847491 A	310 bp	includes part UCR	60
Set34	Shh/HlxB9	CR847479 A	CR847479 A	416 bp	EST shorter than UCR	59/60
Set35	Shh/HlxB9	CR847479 A	CR847479 B	571 bp	EST shorter than UCR	59/60
Set36	Shh/HlxB9	CR847479 B	CR847479 C	1516 bp	Primers in UCR	60
Set37	Irx4	CR846659 A	CR846659 A	436 bp	includes part UCR	60
Set38	Irx4	CR846659 B	CR846659 A	211 bp	includes part UCR	60
Set39	Irx4	CR846776/791/800 A	CR846776/791/800 A	447 bp	includes all 776/791 + part 800	60
Set40	Irx4	CR846776/791/800 A	CR846776/791/800 B	355 bp	includes part 776/791	60
Set41	Shh/HlxB9	CR847031 A	CR847031 A	407 bp	includes part UCR	60
Set42	Shh/HlxB9	CR847031 B	CR847031 A	334 bp	includes part UCR	60
Set43	Lhx2	CR847449 A	CR847449 A	384 bp	includes part UCR	60
Set44	Lhx2	CR847449 A	CR847449 B	411 bp	includes part UCR	60
Set45	Emx2	CR847091 A	CR847091 A	417 bp	does not include UCR	60
Set46	Emx2	CR847091 B	CR847091 A	654 bp	includes part UCR	60
Set47	Emx2	CR847091 B	CR847091 B	267 bp	includes part UCR	60
Set48	Pax6	CR846537 A	CR846537 A	156 bp	includes part UCR	58/60
Set49	Pax6	CR846536 A	CR846536 A	481 bp	includes part UCR	60
Set50	Pax6	CR846536 A	CR846536 B	658 bp	includes part UCR	60
Set51	Pax6	CR846536 B	CR846536 A	427 bp	includes part UCR	60

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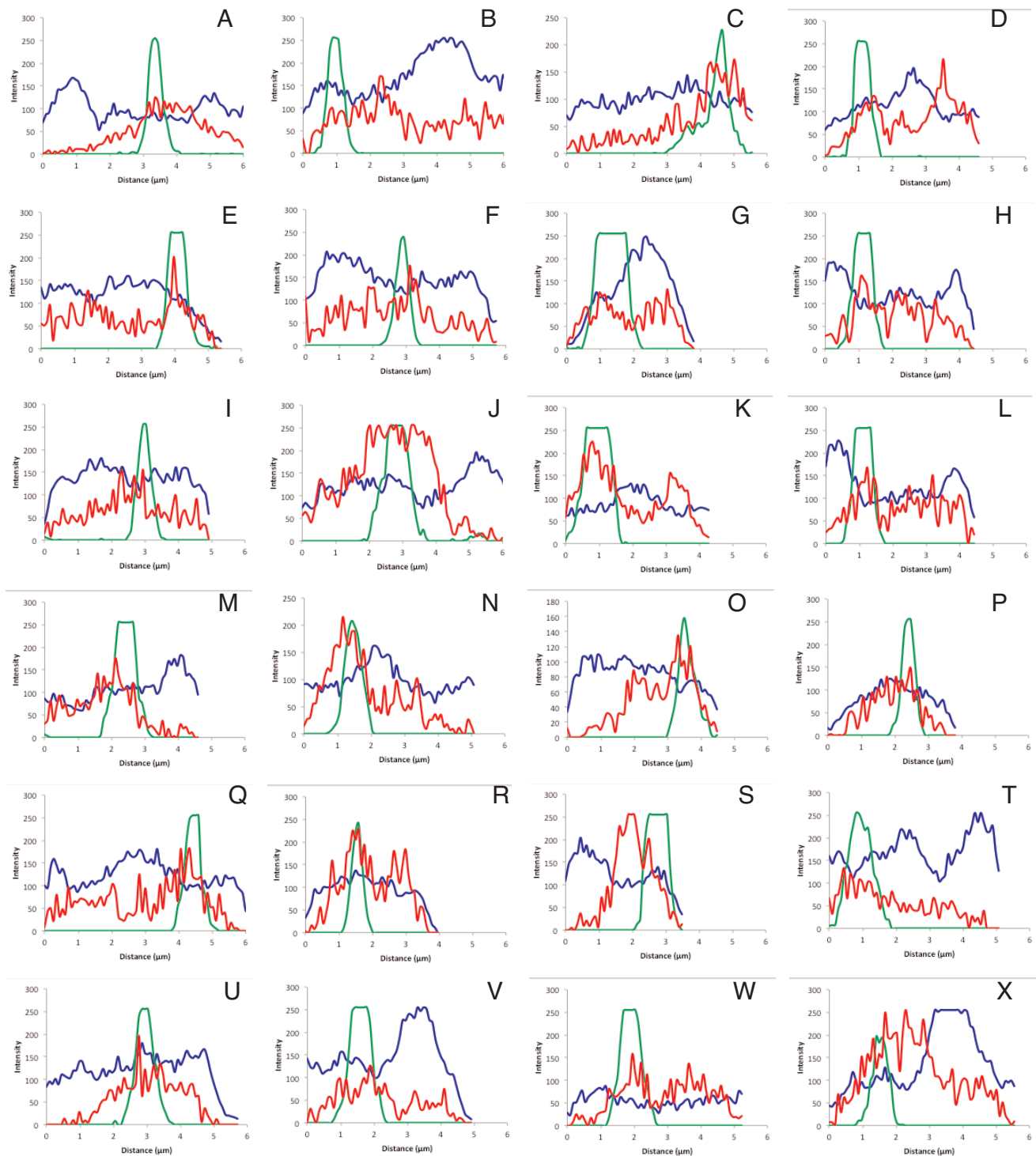
RNAs used in ATPase and remodeling assays

Plasmid	Enz.	Length	Sequence 5'-3' (blue: plasmid sequence-pGEM or pcDNA)
pcDNA-Evf2-(5' end)	NotI	622	GGGAGACCCA --n-- AGCTCGGATC ³² <u>GATCCCCCGG</u> --n-- TCACACTGGC ⁵⁹⁰
pcDNA-Dlx1UR	XhoI	606	GGGAGACCCA --n-- GAATTCGATT ¹⁰⁴ <u>GCTTTGCAAG</u> --n-- ACTGGAGTCG ⁴⁷⁹ <u>TACTACTAGTGAATTCGCGGCCGC</u> ²³
pGEM-28S	SpeI	638	GGGCGAATTG --n-- GAATTCGATT ⁶¹ <u>ACGGACCAAG</u> --n-- AAGAATCA ⁵⁷⁷
pGEM-T Easy	PciI	517	GGGCGAAT --n-- GGAAGAA ⁵¹⁷
tRNA polyA, G			73-94 yeast tRNA SIGMA: R9001 200-500 SIGMA

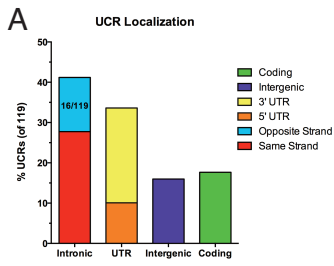
RNAs used in REMSAs: NIR probe and unlabeled competitors

Plasmid	Enz.	Length	Sequence 5'-3' (blue: plasmid sequence-pGEM)
pGEM-Evf2-(UCR)	Sall	206	GGGCGAATTG --n-- AATTCGATTC ⁶² <u>TTTCCTGTT</u> --n-- ACATGAAAAAT ¹¹⁵ <u>TCACTAGTGA</u> --n-- CGCCTGCAGG ²⁹
pGEM-Dlx1UR	Sall	180	GGGCGAATTG --n-- GAATTCGATT ⁶¹ <u>TGTAAAGAGT</u> --n-- TATTCCCCTT ⁹⁰ <u>TCACTAGTGA</u> --n-- CGCCTGCAGG ²⁹
pGEM-28S	BamHI	160	GGGCGAATTG --n-- GAATTCGATT ⁶¹ <u>ACGGACCAAG</u> --n-- CCCGAGGTGG ⁹⁹
pGEM-T Easy	BsrBI	209	GGGCGAATTG --n-- TTGTTATCCG ²⁰⁹
tRNA polyA, U, C, G polyA polyG			73-94 yeast tRNA SIGMA: R9001 200-500 SIGMA 19 IDT 19 IDT

Intensity Profiles of *Evf2*/BRG1/DAPI in individual E13.5 ganglionic eminence nuclei



Cajigas et al Fig S1



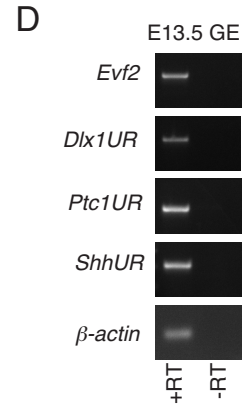
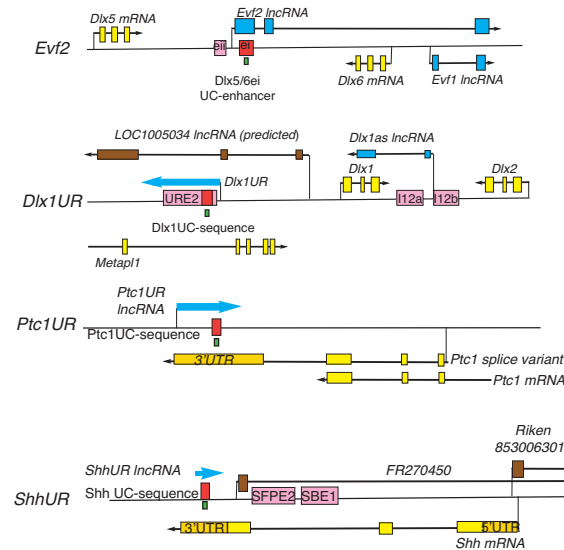
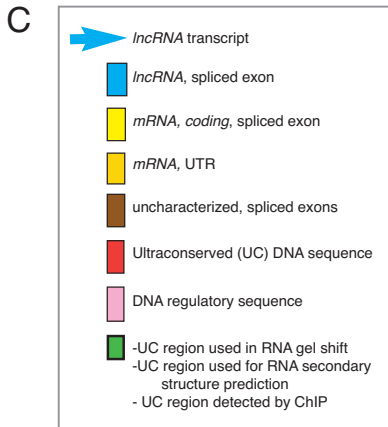
B

	Intron	Intron	UTR	5'	Exon	Unknown or
	Opposite	Sense			Spanning	>10kb away
CR846143			1			
CR846134		1				
CR846130		1				
CR846655					1	
CR847118			1			
CR846271				1	1	
CR847335						1
CR847340						1
CR847338						1
CR846217			1	1		
CR846776						1
CR846791						1
CR846800						1
CR846659						1
CR847029			1			
CR847491				1	1	
CR847031			1			
CR847991				1	1	1
CR847449				1		
CR846537			1			
CR846536			1			
CR847382						1
CR847017				1	1	
CR846172			1			
CR846155			1			
CR846153			1			
CR846158						1
CR846162						1
CR847358						1
CR846189						1
CR846446			1	1		
CR846439			1	1		
CR846444			1			
CR846433						1
CR846431			1			
CR846471				1	1	
CR846470						1
CR846440			1			
CR846128			1	1	1	

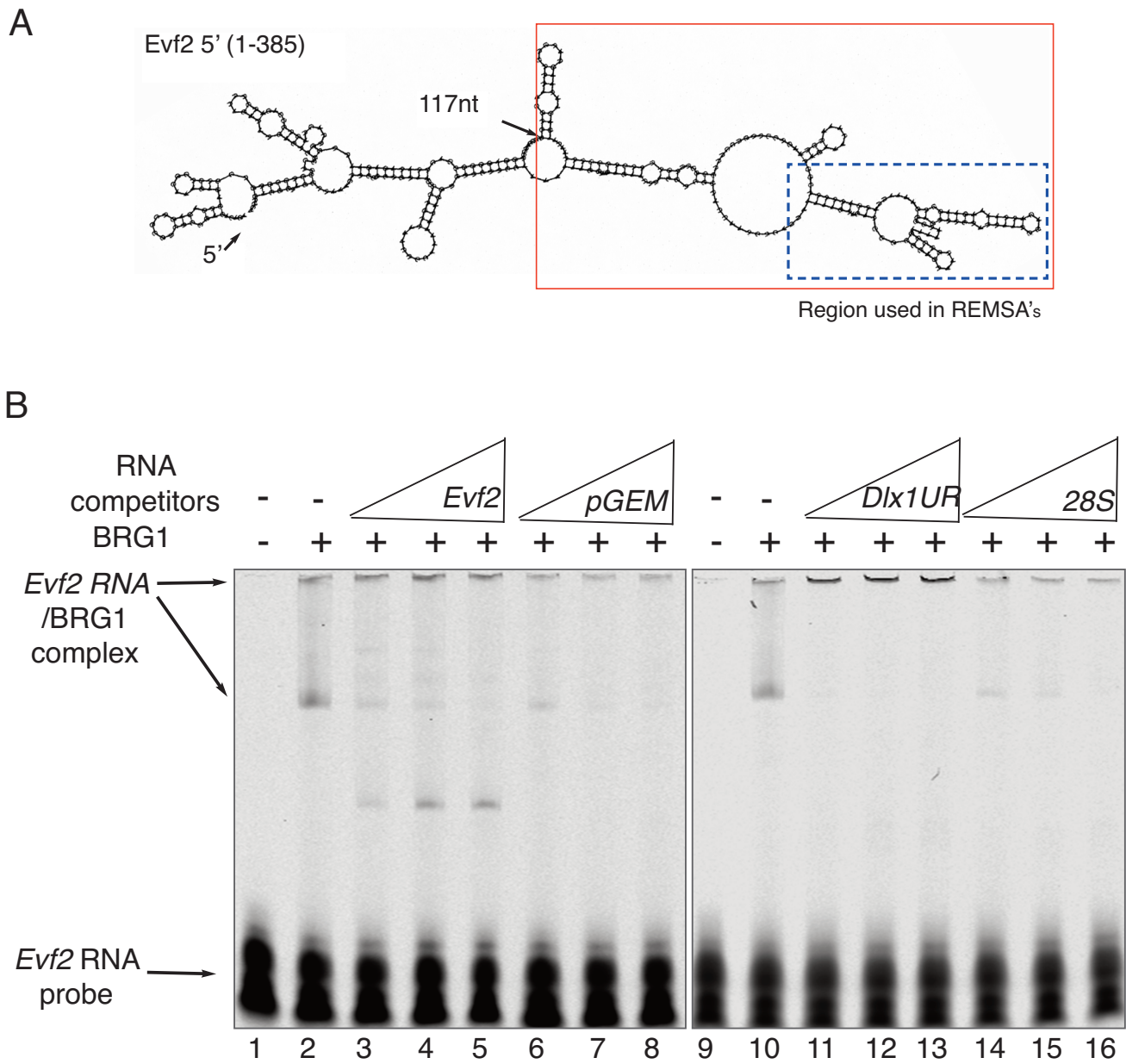
	Intron	Intron	UTR	3'	5'	Exon	Unknown or
	Opposite	Sense				Spanning	>10kb away
CR846242							1
CR846233							1
CR847132						1	
CR847361				1	1		
CR847265				1	1		
CR846515			1				
CR846844				1	1		
CR846852				1	1		
CR846850				1	1		
CR847008				1	1		
CR847147				1	1		
CR847153				1	1		
CR847332				1	1		
CR847334				1	1		
CR846411			1				
CR846561						1	
CR846828							1
CR846823				1	1		
CR846553				1	1		
CR846554				1	1		
CR846554			1				
CR846356				1			
CR846364				1			
CR846340				1			
CR846349							1
CR847297				1			
CR846383							1
CR846382							1
CR846947					1	1	
CR846391					1		
CR846829							1
CR846394					1		
CR847225					1		
CR847235					1		
CR847458				1	1		
CR847421					1	1	
CR846891					1		
CR846885					1		
CR846908					1		

TOTAL UCRs with EST Hit

Category	Count	%
Total UCRs with EST Hit	119	
Total ESTs in Intron Opposite Direction	16	13.45%
Total ESTs in Intron Same Direction	33	27.73%
Total ESTs in Intron	49	41.18%
Total ESTs in UTRs	40	33.61%
Total ESTs in 3' UTRs	28	23.53%
% of UTRs		70.00%
Total ESTs in 5' UTRs	12	10.08%
% of UTRs		30.00%
Total ESTs with coding sequence	21	17.65%
Unknown or >10kb from gene sequence	19	15.97%



Cajigas et al Fig S2



Cajigas et al. Fig S3