**EN-08-0779v2 Supplementary Information**

**Supplementary Note 1 Semi-quantitative PCR**

Prior to carrying out the amplification procedure, optimal primer concentrations for amplification of rEap1 and cyclophilin cDNAs (1 and 0.6 μM, respectively) were determined, in addition to the linear range of the PCR reactions. Cyclophilin mRNA is constitutively expressed and thus serves as an internal standard. The sense rEap1 primer used (5'-AGCCCCAACTCATCCTCAG-3') corresponds to nt 607-625 in r*Eap1* mRNA (GenBank NM_001012470); the antisense primer (5'-ACGCTCCTGGTCTGTGCTC-3') is complementary to nt 969-987. The sense cyclophilin primer (5'-ACGCCGCTGTCTCTTTTC-3') corresponds to nt 9-26 in rat cyclophilin mRNA (GenBank M19533) and the antisense primer (5'-CTTGCCACCAGTGCCATTAT-3') is complementary to nt 251-270. The PCR program employed consisted of an initial activation step of 15 min at 95 C, and 34 cycles of denaturing at 94 C for 30 sec, annealing at 60 C for 30 sec and 1 min extensions at 72 C, followed by a final extension of 10 min at 72 C. The primers were designed using Primer Select software (DNASTAR Lasergene software v5.08, DNASTAR Inc., Madison, WI).

**Supplementary Note 2 Real-time PCR**

This procedure was carried out after diluting 1:50 the reverse transcription reaction (500 ng total RNA in 20 μl) and using 2 μl of this dilution for PCR amplification. rEap1 primers and the fluorescent probe used were designed to target a segment comprised within a cloned r*Eap1* cDNA described in the **Material and Methods** section of the manuscript. The primers were selected with the assistance of the Primer Express program (Perkin Elmer Applied Biosystems, Foster City, CA). The primer sequences for *rEap1* were: 5'forward (5'-ACTGTATCCTCTGCTCCGTCATC-3') corresponding to nt 883-905 in r*Eap1* mRNA (GenBank NM_001012470) and 3' reverse (5'-ACCGATCCAGGCCTCTTACC-3'), complementary to nt 946-965. The internal fluorescent oligodeoxynucleotide probe (Applied Biosystems) had a sequence (5'-CCCACCTCTGCCACTGTCGACGAG-3') complementary to nt 909-932 in r*Eap1*. Real-time PCR reactions were performed in a total volume of 10 μl, each reaction containing 2 μl of the diluted reverse transcribed sample or 2 μl of the reverse transcribed sense RNA standard, 5 μl TaqMan Universal PCR Master Mix (Applied Biosystems), 250 nM of each gene-specific and ribosomal fluorescent probes, 300 nM of each gene specific primer, and 10 nM of each ribosomal primer. The real-time PCR program used consisted of an initial annealing period of 2 min at 50 C, followed by 10 min of denaturing at 95 C, and 40 cycles of 15 sec at 95 C and 1 min at 60 C.

**Supplementary Note 3 Construction of a rEap1-luciferase reporter plasmid**

To PCR amplify a DNA fragment containing the 5' flanking region of the *rEap1* gene we used PCR primers (sense: 5'-ATACTCGAGAGCCGGCTGTAATAGTGGTCATA-3'; antisense: 5'-ATACTCGAGCCCGGTGCCCAGGTCCAATCTT-3') that were modified to contain a XhoI restriction site at each end of the PCR product. The DNA fragment was PCR-amplified from rat tail DNA (isolated by the Proteinase K method) with the Failsafe PCR PreMix Selection kit (Epicentre, Madison, WI) in a 100 μl reaction containing 50 μl of buffer D, 4 μl of each primer (50 μM), 0.5 μg DNA and 4 μl of enzyme. The PCR program consisted of a first denaturing step of 2 min at 95 C, followed by 35 cycles of 30 sec at 95 C, 1 min at 57 C and 3.5 min at 72 C, followed by a final extension of 10 min at 72 C. The PCR product obtained was first cloned into pGEM-T (Promega), digested with Xho I, blunted and ligated into pGL-3-Basic (Promega). The sequence of the subcloned DNA fragment was verified by sequencing.

**Supplementary Note 4 Transfection Assays**

Native C6 cells (C6) were grown in Dulbecco's modified Eagle medium (DMEM, Sigma Chemicals, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS, Hyclone, Logan, UT), 100 μg/ml penicillin, and 100 μg/ml streptomycin. C6α and C6β cells were grown in the same medium supplemented with Hygromycin (200 μg/ml, Invitrogen, Carlsbad, CA). For promoter assays, cells were seeded in 12 well plates (at a 120,000 cells/well), and grown in DMEM without phenol red or antibiotics, containing 10% charcoal-stripped FBS (Hyclone) and glutamine (20 mM). Twenty-four hours after seeding, Lipofectamine 2000 (Invitrogen) was premixed for 30 min with the various reporter gene constructs at a ratio of 1 μg DNA to 2 μl transfection reagent before the mixture was added to the cells. Transfection efficiency was normalized by co-transfecting cells with 250 ng/well of CMV-Sport-β-gal (Invitrogen). The total amount of DNA transfected was kept constant at 1 μg by adding the appropriate amount of pCDNA3.1 to each well. The transfection medium was replaced after 5 h by fresh DMEM-10% FBS without phenol red. The cells were harvested 48 h after transfection and assayed for luciferase and β- galactosidase, as reported {3018, 5761}. Basal activity of the r*Eap1*-pGL3 construct was first tested in native C6 cells, transfecting the cells with increasing amount of the *Eap1* construct (100, 250, 500 and 1000 ng/ml).

## Supplementary Note 5 Immunohistofluorescence

The EAP1 immunoreaction was developed by incubating the sections with Alexa 594-labeled donkey antirabbit IgG (1:250; Invitrogen). The ERα reaction was developed with biotinylated horse antimouse gamma globulin (1:250, Vector Laboratories, Burlingame, CA) for 1 h at room temperature, followed by incubation with Streptavidin- Alexa 488 (1:500; Invitrogen). Fluorescent images were acquired as reported {5761} using a Marianas™ digital imaging workstation (Intelligent Imaging Innovations, Denver, CO) with a 40x C-apochromat NA1.2 objective. The Marianas workstation is equipped with a Zeiss Axiovert 200M microscope (Zeiss, Thornwood, NY) and a motorized stage (API, Eugene, OR).