

## Supplementary material

### Legends for supplementary figures

**Fig.S1.** (A) Sequence alignment of XRbm9, hRbm9 (isolated in this study) and RTA [1]. Note that XRbm9 and hRbm9 used in this study carry two different C-terminal sequences (broken line). (B) Sequence alignment between the additional exon, which was isolated during the cloning of XRbm9 and hRbm9. This sequence is present in the putative amino-acid sequence of the *X.tropicalis* Rbm9.

**Fig.S2.** (A,B) XRbm9 antibody specificity. (A) Immunoprecipitates of *in vitro* translated [<sup>35</sup>S]methionine-labeled XRbm9 protein with control IgG, XRbm9 antibody alone or preincubated with XRbm9 antigen were analyzed by autoradiography. Preincubation of the XRbm9 antibody with the antigen abrogates its ability to immunoprecipitate the *in vitro* translated XRbm9 protein. (B) Oocytes extracts overexpressing XRbm9 or HA-tagged XRbm9ΔN were immunoprecipitated and analyzed by Western blotting as indicated. The XRbm9 antibody specifically precipitates XRbm9 or the HA-tagged XRbm9ΔN proteins overexpressed in oocytes.

**Fig.S3.** XRbm9 antibody injection does not affect cytoplasmic polyadenylation. Same experiment as the one described in Fig.5 with XRbm9, XGld2 or CPEB antibodies.

**Fig.S4.** (A) XGld2 antibody characterization. Immunoprecipitates of *in vitro* synthesized (35S)methionine-labelled XGld2Δ4 protein with control IgG, XGld2 antibody alone or preincubated with XGld2 antigen were analyzed by autoradiography. Preincubation of the XGld2 antibody with the antigen abrogates its ability to immunoprecipitate the *in vitro* translated XGld2Δ4 protein. XGld2Δ4 is an alternatively spliced variant isolated in stage VI oocytes that lacks exon 4. (B) Predicted amino acid sequences of XGld4 and XGld2Δ4 that were isolated in this study.

## Materials and Methods

### Cloning *Xenopus* Rbm9

With the hRbm9 cDNA sequence isolated during the two-hybrid screen as the query sequence, we ran a BLAST search on databases from the *X. laevis* EST project (<http://www.sanger.ac.uk>). This search generated multiple overlapping ESTs that yielded a complete ORF. Stage VI oocyte

total RNA was used to perform an oligo(dT) primed reverse transcription using the Superscript<sup>TM</sup>II reverse transcriptase (Invitrogen). PCR using the following primers: 123: 5'-CCCTTTCCTGTTAGCAGTGTG-3' and 120: 5'-GGGACAATAGGCTTACGTC-3' was performed and the amplified PCR products were cloned into a TA cloning vector (Invitrogen) and sequenced. An alternatively spliced exon was also isolated in the course of the XRbm9 cloning (Fig.S1).

### **Cloning of XGld2 and hGld2**

The CeGld2 cDNA sequence was used as a reference in our BLAST search of databases from the *X. laevis* EST project (<http://www.sanger.ac.uk>). This search yielded multiple overlapping ESTs that produced a complete ORF. Stage VI oocyte total RNA was used to perform an oligo(dT) primed reverse transcription using the Superscript<sup>TM</sup>II reverse transcriptase (Invitrogen). PCR using the following primers 74: 5'-GTCGCTGTGTTGTTCTGTCAGGC-3' and 75: 5'-GGCCACCGTTTTAGCATTCTCCC-3' was performed and the amplified PCR products were cloned into a TA cloning vector (pCRII) (Invitrogen) and sequenced. The longest clone corresponded to the XGld2 cDNA described in Barnard et al. (2004). The shortest corresponded to an alternatively spliced form of XGld2 missing exon 4 (XGld2Δ4, see Fig.S4). A BLAST search of the human genome database was conducted by using the XGld2 coding sequence to identify homologous human cDNAs. Primers encompassing a putative ORF were designed as follows: 89: 5'-ATCGATATGTTCCCAAACCTCAATTTGGGTCG-3' and 90: 5'-TAGAGACCAGTTATCTTTTCAG-3'. Oligo(dT) primed cDNA from SW80 cell line RNA was used to perform a PCR using the above primers. The PCR products were cloned into a TA cloning vector (Invitrogen) and sequenced. Three human cDNAs corresponding to those described in Rouhana et al. (2005) were isolated. The cDNA used for the two-hybrid screen was the alternatively spliced variant lacking exon 8 (hGld2Δ8).

### **DNA constructs**

#### **XGld2:**

Expression vectors:

pCS2 XGld2, pCS3 XGld2, pCSH XGld2: XGld2 ORF from the pCRII XGld2 was inserted into the ClaI-EcoRI sites of the pCS2+ vector, pCS3 vector (in frame with the myc tag) and pCSH vector (in frame with the HA tag [2]).

pCS2 XGld2Δ4: XGld2 ORF from the pCRII XGld2Δ4 was inserted into the Cla1-EcoR1 sites of the pCS2+ vector.

Two-hybrid vectors:

pGBT9 XGld2: XGld2 ORF from the pCRII XGld2 was inserted into the Cla1-Kpn1 sites of the pBSKS+ vector. XGld2 ORF from pKS XGld2 was then inserted in frame with the Gal4 DNA binding domain (Gal4BD) at the EcoR1 site of the pGBT9 vector. pGBT9 XGld2N: The EcoR1-Pst1 fragment (XGld2 amino-acids 1 to 149) from the pKS XGld2 was inserted in frame with the Gal4BD into the pGBT9 vector. pGBT9 XGld2Δ4 and pGBT9 XGld2Δ4N were generated from the pCRII XGld2Δ4 as described for XGld2 full length.

### **hGld2:**

Two-hybrid vectors:

pGBT9 hGld2: hGld2 ORF from the pCRII hGld2Δ8 was inserted into the EcoR1 site of the pGBT9 vector in frame with the Gal4BD. pGBT9 hGld2N: The EcoR1-Pst1 fragment (hGld2Δ8 amino-acids 1 to 185) from the pCRII hGld2Δ8 was inserted in frame with the Gal4BD into the pGBT9 vector. pGBT9 hGld2C: The Pst1 fragment (hGld2Δ8 amino-acids 184 to 480) from the pCRII hGld2Δ8 was inserted in frame with the Gal4BD into the pGBT10 vector.

### **XRbm9:**

pCRII XRbm9ORF: XRbm9 ORF from the ATG to the stop codon of the protein was generated by PCR using the following primers: 122: 5'-ATCGATATGGCAGATGCTGTAATGTC-3' and 120 as above. The amplified PCR products were cloned into a TA cloning vector (Invitrogen) and sequenced. pCRII XRbm9ΔN: XRbm9ΔN fragment corresponding to amino-acid 55 to 411 was amplified by PCR with the following primers: 113: 5'-ATCGATATGAGTATGCCAGTGTCCCAAGC-3' and 120 as above. The amplified PCR products were cloned into a TA cloning vector (Invitrogen) and sequenced.

Expression vectors:

pCS2 XRbm9: XRbm9 ORF from the pCRII XRbm9ORF was inserted into the Cla1-EcoR1 sites of the pCS2+ vector. pCSH XRbm9ΔN: XRbm9ΔN fragment from the pCRII XRbm9ΔN was inserted into the Cla1-EcoR1 sites of the pCSH vector [2] in frame with the HA tag.

Two-hybrid vectors:

pAD XRbm9: XRbm9 ORF from the pCRII XRbm9ORF was inserted in frame with the Gal4 activation domain (Gal4AD) into the EcoR1 site of the pADGal4 vector. pAD XRbm9ΔN:

XRbm9 $\Delta$ N fragment from the pCRII XRbm9 $\Delta$ N was inserted in frame with the Gal4AD into the EcoR1 site of the pADGal4 vector.

MS2 fusion protein:

cDNA encoding XRbm9 was cloned as a PCR product using the following primers: 5'-TGCTAGCATGGCAGATGCTGTAATG-3' and 5'-CCTCGAGTCAGTACGGAGCAAATCG-3' containing NheI and XhoI restriction sites (underlined) into the NheI-XhoI restricted pMSP vector.

### **hRbm9**

Expression vectors:

pCSH hRbm9: hRbm9 cDNA isolated during the two-hybrid screen (hRbm9 amino-acid 2 to 401 in the pADGal4 vector) was inserted into the EcoR1-XhoI sites of the pCSH vector in frame with the HA tag. pCSH hRbm9 $\Delta$ N: hRbm9 $\Delta$ N fragment (hRbm9 amino-acids 48 to 401) from the pADGal4 hRbm9 was inserted into the EcoR1-XhoI sites of the pCSH vector in frame with the HA tag.

Two-hybrid vectors:

pAD hRbm9 $\Delta$ N: hRbm9 fragment corresponding to amino-acids 48 to 401 from the pADGal4 hRbm9 was inserted into the EcoR1-SmaI sites of the pBSKS+ vector to generate pKS hRbm9 $\Delta$ N. The EcoRV-SmaI fragment from this plasmid was inserted in frame with the Gal4AD into the SmaI site of the pADGal4 vector. pAD hRbm9 48-269: hRbm9 fragment corresponding to amino-acids 48 to 269 from the pADGal4 hRbm9 was inserted into the EcoR1-PstI sites of the pBSKS+ vector to generate pKS hRbm9 48-269. The EcoRV-PstI fragment from this plasmid was inserted in frame with the Gal4AD into the SmaI-PstI sites of the pADGal4 vector. pAD hRbm9 269-350: hRbm9 fragment corresponding to amino-acids 269 to 350 from the pADGal4 hRbm9 was inserted in frame with the Gal4AD into the PstI site of the pADGal4 vector.

### **References**

1. Norris, J.D., Fan, D., Sherk, A. and McDonnell, D.P. (2002). A negative coregulator for the human ER. *Mol Endocrinol* **16**, 459-68.
2. Rouget, C., Papin, C. and Mandart, E. (2006). Cytoplasmic CstF-77 protein belongs to a masking complex with cytoplasmic polyadenylation element-binding protein in *Xenopus* oocytes. *J Biol Chem* **281**, 28687-98.