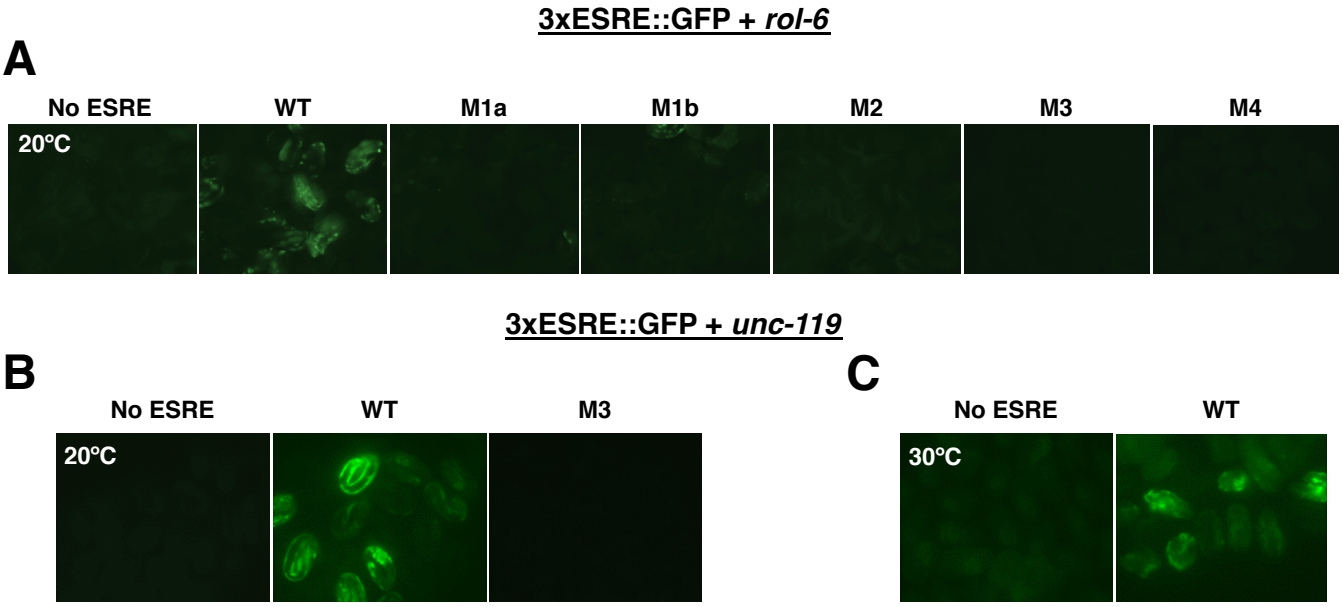


FIG S1 Mutational analysis of the ESRE regulatory element. (A) Representative images of wild-type embryos containing a 3xESRE::GFP extrachromosomal array (WT), a no-ESRE GFP control, or 3xESRE variants (M1a, M1b, M2, M3, and M4) along with the *rol-6(gf)* marker at 20ºC. Exposure times for all images in A were identical to those used in Figure 1A. (B) *unc-119(ed3)* embryos with arrays containing wild-type *unc-119* along with the wild-type 3xESRE::GFP (WT), the M3 variant, or the no-ESRE GFP reporter at 20ºC. (C). *unc-119(ed3)* embryos with arrays containing wild-type *unc-119* along with the wild-type 3xESRE::GFP (WT) or no-ESRE reporter at 30ºC. Exposure times for images in B and C were identical to each other but were longer than exposure times for A. Strains carrying the 3xESRE::GFP + *unc-119*$^{+}$ arrays showed constitutively low levels of reporter expression in neurons and body wall muscles at 20ºC, similar to strains carrying 3xESRE::GFP + *rol-6(gf)* at 20ºC. Notably, the *unc-119*$^{+}$ arrays did not show increased expression at 30ºC, suggesting that sequences within the *rol-6* gene are permissive for stress-inducible expression of 3xESRE::GFP arrays or that sequences within *unc-119* prevent stress-inducible expression.

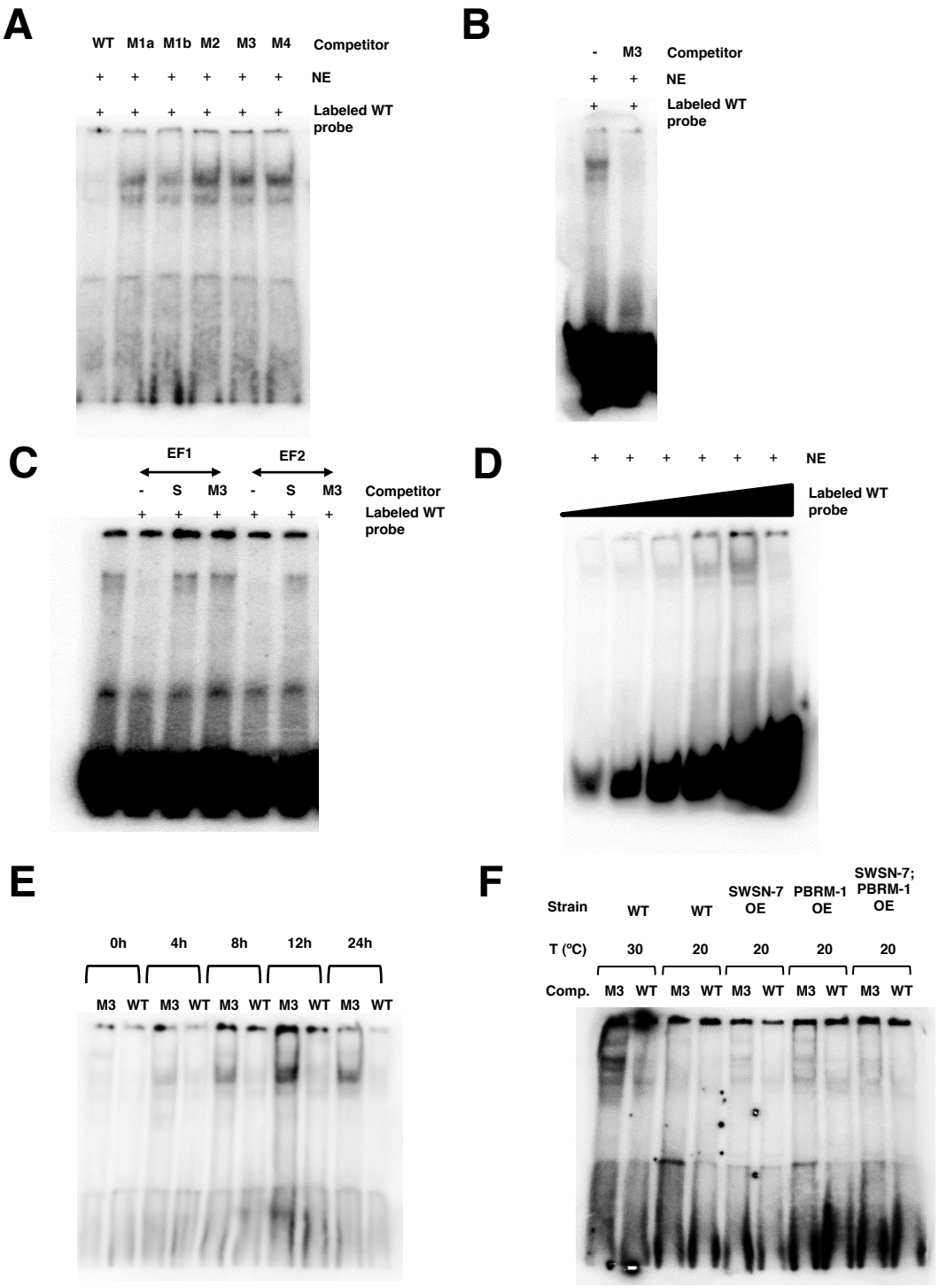


FIG S2 Additional EMSA experiments and images. (A) Image of entire gel from Figure 1C. (B) ESRE-binding activity observed using crude nuclear extracts prepared from stressed (30ºC) wild-type worms in the presence of nonspecific competitor poly dI-dC (-) or wild type competitor (WT). (C) EMSA performed using partially purified nuclear extracts from stressed worms (EF1 and EF2) in the presence of nonspecific competitor poly dI-dC, wild type competitor or mutant competitor containing three altered nucleotides (M3). (D) Estimation of DNA-binding affinity and TF concentration using Scatchard analysis. Identical concentrations of nuclear extract from stressed worms were incubated with increasing amounts of labeled probe in the presence of poly dI-dC. The Kd for the ESRE-binding activity was determined to be 0.43-0.77 nM, whereas the concentration of the ESRE-binding protein was estimated to be 0.39-0.5 nM. (E) Image of entire gel from Figure 2A. (F) Image of entire gel from Figure 10B.

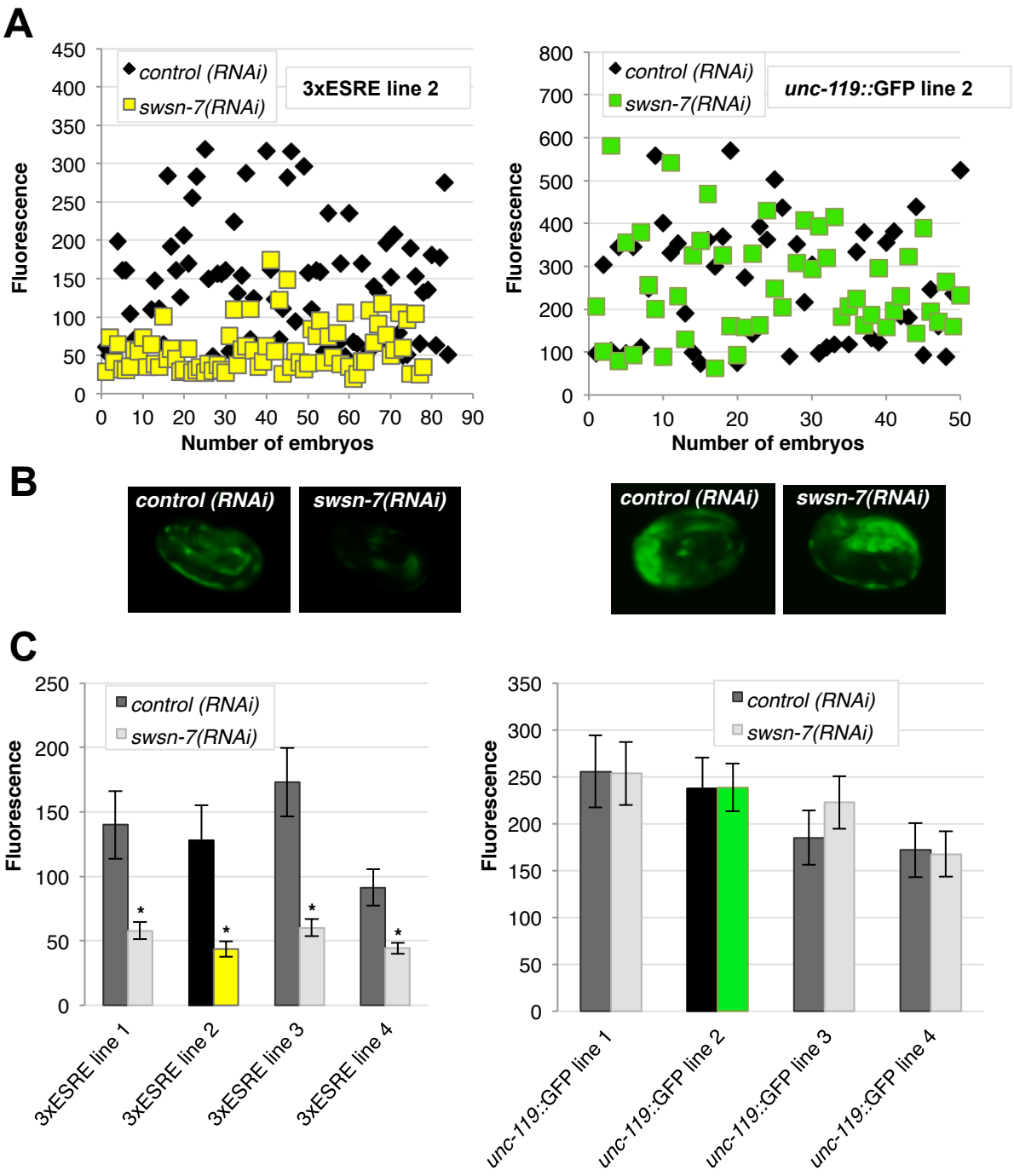


FIG S3 Knockdown of *swsn-7* attenuates 3xESRE::GFP expression. (A) Fluorescence intensities of individual embryos from representative strains carrying either an *unc-119*$^{+}$-marked 3xESRE::GFP array (left) or an *unc-119*::GFP array (right). Strains were pretreated with either *control (RNAi)* or *swsn-7 (RNAi)*. (B) Representative images of embryos from A. (C) Quantification of GFP fluorescence intensities in wild-type embryos from multiple independent *unc-119*$^{+}$-marked 3xESRE::GFP and *unc-119*::GFP lines pretreated with *control (RNAi)* or *swsn-7(RNAi)*. Error bars indicate 95% CIs. Statistical analysis was done using Student's t-test; asterisks indicate statistical significance ($p < 0.001$) relative to control ($n \geq 50$).

A

RNAi Dilution	Total embryos	Hatched embryos (%)	Arrested L1s (%)
control	158	98.7	0
swsn-7	146	55.5	91.4
1:2	136	73.5	74
1:4	129	73.6	43.2
1:8	144	98.6	12.7
1:16	129	99.2	0
1:32	154	98.7	0

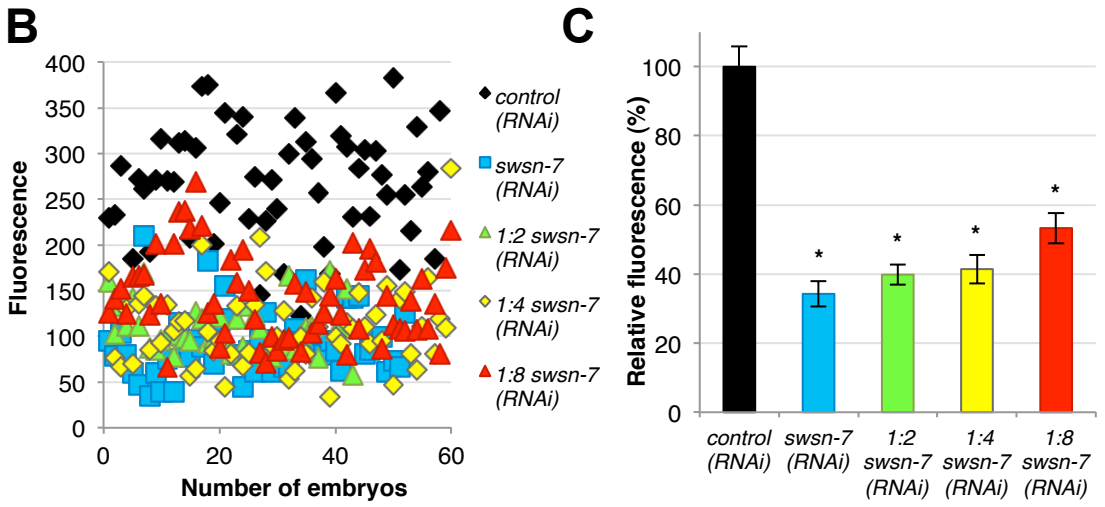


FIG S4 Effects of *swsn-7(RNAi)* on 3xESRE::GFP expression are not due to reduced viability of embryos. (A) Stepwise *swsn-7 (RNAi)* dilutions resulted in a gradual decrease in both embryonic lethality and L1 arrest. (B) Fluorescence intensities of individual heat-shocked (30ºC) embryos that contained a 3xESRE::GFP array for each of the tested *swsn-7(RNAi)* dilutions. (C) Quantification of fluorescence intensities for each of the dilutions. Results are normalized to the intensity of the control, which was arbitrarily set at 100%. Error bars indicate 95% CIs. Statistical analysis was done using Student's t-test; asterisks indicate statistical significance ($p < 0.001$; $n = 40–60$).

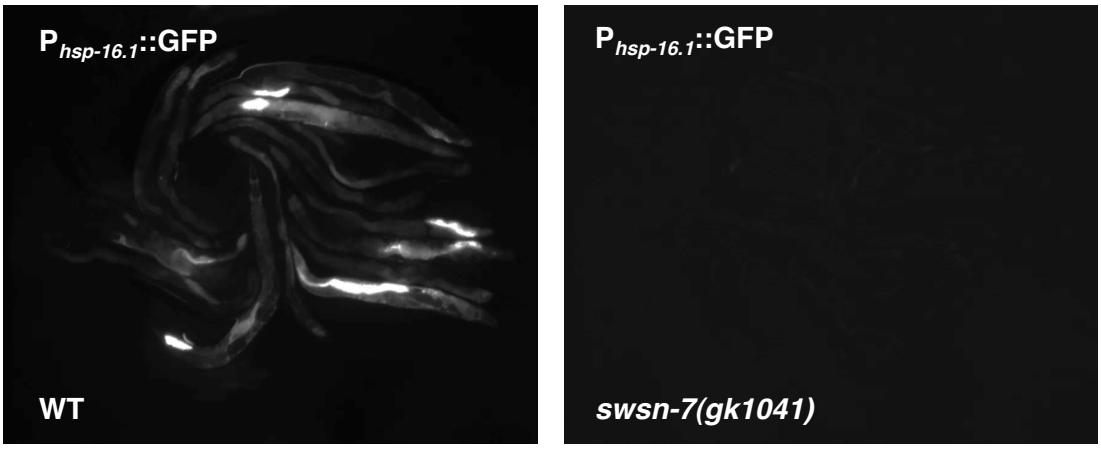
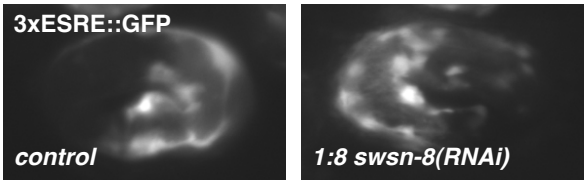


FIG S5 Knockdown of *swsn-7* attenuates ESRE-mediated transcription following exposure to hypoxia. Wild-type (WT) and *swns-7(gk1041)* L4 larvae and adults carrying the $P_{hsp\text{-}16.1}$::GFP reporter were incubated under low oxygen conditions for 6 hours in M9 media without shaking and then allowed to recover for 30 minutes on feeding plates (63). Although hypoxia leads to a weaker induction of $P_{hsp\text{-}16.1}$::GFP in wild-type animals as compared with heat shock (Fig. 4), expression of $P_{hsp\text{-}16.1}$::GFP was notably weaker in *swns-7(gk1041)* mutants than in wild type in response to hypoxia.

A

Dilutions	Total embryos	Unhatched embryos (%)
control	95	2.1
1:2	144	73.6
1:4	130	50.8
1:8	151	27.8

B



C

FIG S6 Effects of *swsn-8(RNAi)* on 3xESRE::GFP and $P_{hsp\text{-}16.2}$::GFP expression. (A) Stepwise *swsn-8 (RNAi)* dilutions resulted in a gradual decrease in *swsn-8*-associated embryonic lethality. (B) Representative GFP fluorescence images of heat-shocked (30ºC) embryos containing a 3xESRE::GFP reporter following *control (RNAi) or 1:8 swsn-8(RNAi)* treatment. (C) Fluorescence images of heat-shocked animals containing a $P_{hsp\text{-}16.2}$::GFP reporter treated with *control (RNAi)* or several dilutions of *swsn-8(RNAi)*. Note that *swsn-8(RNAi)* failed to reduce expression levels of both ESRE reporters despite inducing embryonic lethality.

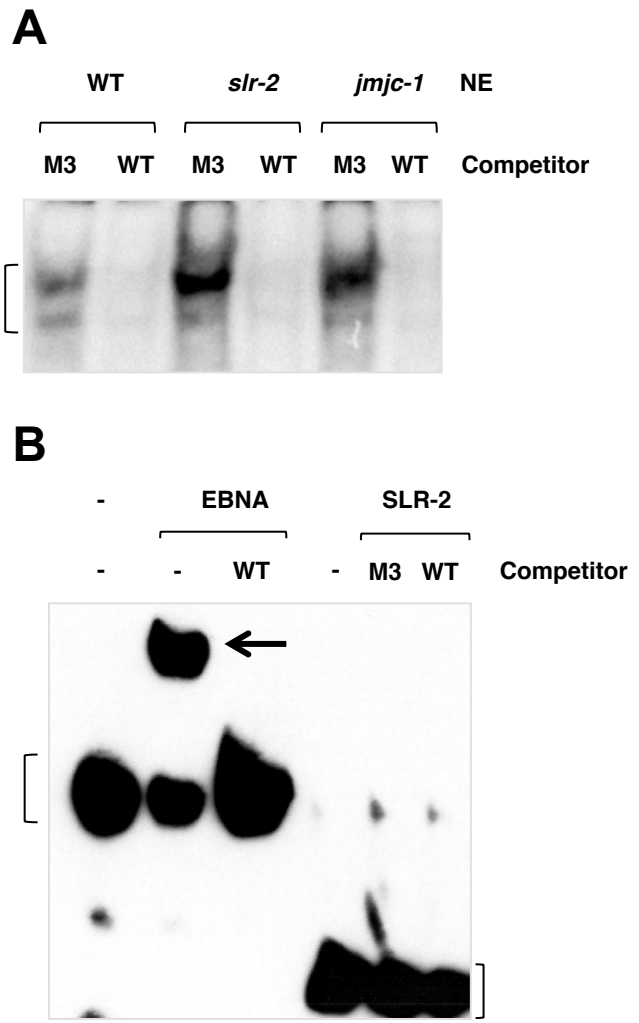


FIG S7 SLR-2 and JMJC-1 do not bind the ESRE. (A) ESRE-binding activity detected by EMSA performed with nuclear extracts (NE) from wild-type (WT), *slr-2(ku297)*, and *jmjc-1(tm3525)* null mutants exposed to heat shock (30ºC) for 12 h. Bracket indicates location of the shifted band(s). WT indicates unlabeled specific competitor and M3 unlabeled nonspecific competitor. We note that the apparent increased intensity of the shifted band in the mutant backgrounds was not reproducibly observed. (B) EMSA carried out using purified SLR-2 protein expressed from *E. coli*. EBNA (Epstein-Barr nuclear antigen 1) was used as a positive control for protein-DNA binding. The square brackets indicate unbound labeled probe. Black arrow indicates the shifted EBNA band. No shift was observed with recombinant SLR-2 and the ESRE probe.