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GNL3L Inhibits Estrogen Receptor-Related Protein Activities by Competing for Coactivator Binding

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Summary

Guanine-nucleotide binding protein 3-like (GNL3L) is the closest homologue of a stem cell-enriched factor nucleostemin in vertebrates. They share the same yeast orthologue, *Grn1p*, but only GNL3L can rescue the growth-deficient phenotype in *Grn1p*-null yeasts. To determine the unique function of GNL3L, we identified estrogen receptor-related protein-γ (ERRγ) as a GNL3L-specific binding protein. GNL3L and ERRγ are coexpressed in the eye, kidney and muscle, and co-reside in the nucleoplasm. The interaction between GNL3L and ERRγ requires the intermediate domain of GNL3L and the AF2-domain of ERRγ. Gain- and loss-of-function experiments show that GNL3L can inhibit the transcriptional activities of ERR genes in a cell-based reporter system, which does not require the nucleolar localization of GNL3L. We further demonstrate that GNL3L is able to reduce the steroid receptor coactivator (SRC) binding and the SRC-mediated transcriptional coactivation of ERRγ. This work reveals a novel mechanism that negatively regulates the transcriptional function of ERRγ by GNL3L through coactivator competition.

Keywords

ERR; Estrogen Receptor; GNL3L; Nucleolus; Nucleostemin; SRC

Introduction

Nucleostemin and its homologues, guanine-nucleotide binding protein 3-like (GNL3L) and Ngp1, constitute a subfamily of GTP-binding proteins featured by their nucleolar distribution and a unique domain of circularly permuted GTP-binding motifs, where the G4 motif is located N-terminally to the G1, G2, and G3 motifs (Daigle et al., 2002; Leipe et al., 2002). Nucleostemin is enriched in the embryonic, mesenchymal, and neural stem cells, adult testes, and several types of human cancers (Baddoo et al., 2003; Kafienah et al., 2006; Tsai and McKay, 2002). It plays a role in maintaining the continuous proliferation of neural stem cells (Tsai and McKay, 2002) and in regulating the protein stability of telomeric repeat-binding factor 1 (TRF1) (Zhu et al., 2006). Targeted deletion of nucleostemin leads to early embryonic lethality in homozygous nucleostemin-null embryos (Beekman et al., 2006; Zhu et al., 2006) and premature senescence of heterozygous nucleostemin-null mouse embryonic fibroblast cells (Zhu et al., 2006).

Phylogenetically, nucleostemin is most closely related to GNL3L in vertebrates. They share the same yeast orthologue, *Grn1p* in *Schizosaccharomyces pombe* and *Nug1p* in

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Saccharomyces cerevisiae. *Grn1p* is involved in the processing of 35S pre-ribosomal RNA (rRNA), the nuclear export of Rpl25, and the maintenance of cell growth (Du et al., 2006). Mutation in *Nug1p* inhibits the export of 60S subunit from the nucleolus (Du et al., 2006; Kallstrom et al., 2003). Although the yeast orthologue of nucleostemin and GNL3L displays general activities in growth and ribosome biogenesis, rodent nucleostemin and GNL3L are distinctively expressed in different tissues. Furthermore, only human GNL3L, but not nucleostemin, can rescue the *Grn1p*-deficient growth phenotype in fission yeasts (Du et al., 2006). These results suggest that nucleostemin and GNL3L have evolved specific properties in vertebrates, and become functionally diverged from each other and from *Grn1p*. By comparison, GNL3L retains more characteristics of *Grn1p* than does nucleostemin.

GNL3L bears 28% protein identity and 39% protein similarity to nucleostemin in mice. Very little is known about its function in vertebrates. To delineate the distinct activity of GNL3L, we looked for proteins that might interact with GNL3L but not with nucleostemin. We first identified estrogen receptor-related protein-γ (ERRγ) as a GNL3L-binding protein by a yeast two-hybrid screen, and confirmed this interaction by affinity-binding and coimmunoprecipitation assays. ERRγ belongs to a subfamily of the nuclear receptor superfamily. The ERR gene family consists of three members, ERRα, ERRβ, and ERRγ, that most resemble estrogen receptor-α (ERα). Like ERα, the ERR proteins contain functionally separable structures that include an AF1 domain (or A/B region), a DNA-binding domain (DBD, or C region) with two zinc fingers, a hinge region (D), and a ligand-binding domain (LBD, or E/F region) with an AF2 domain at the C-terminal end. The LBD is involved in ligand binding, receptor dimerization, and coactivator binding. The AF2 domain is required for the ligand-dependent activation function. ERRα and ERRγ are found in the brain, muscle, heart, kidney, and adipose tissues (Bonnelye et al., 1997; Giguere et al., 1988; Hong et al., 1999). ERRβ is expressed in the eye, heart, kidney, cerebellum, and testis (Bookout et al., 2006). The functions of the ERR family genes are implicated in many aspects of embryogenesis and tumorigenesis. Mice deficient in ERRα exhibit reduced body weight and peripheral fat deposit, and are resistant to obesity induced by a high-fat diet (Luo et al., 2003). ERRβ-null mice display placentation defects, consistent with its role in the proliferation and differentiation of trophoblastic cells (Luo et al., 1997). In humans, the expression level of ERRα correlates with poor prognosis in ovarian and breast cancers (Ariazi and Jordan, 2006; Suzuki et al., 2004). Conversely, ERRγ is a favorable indicator for human ovarian tumors (Sun et al., 2005). Although the ERR family genes are capable of binding the estrogen response element (ERE), they are different from ERα in that their transcriptional activity and coactivator binding do not require ligand binding (Giguere et al., 1988; Hong et al., 1999; Kallen et al., 2004), which leaves open the question of whether the activities of the ERR family genes are constitutively active or dynamically regulated.

In this manuscript, we uncover a GNL3L-mediated pathway that regulates the transcriptional activity of the ERR family genes. We show that only GNL3L, but not nucleostemin or Ngp1, can interact with the ERR family genes. Coexpression of GNL3L is able to inhibit the transcriptional activities of the ERR family genes. Conversely, knocking down the endogenous expression of GNL3L increases the ERR-mediated transactivation. Furthermore, GNL3L can compete with steroid receptor coactivators (SRCs) for their ERRγ binding and block the SRC-mediated coactivation of ERRγ. Our study reveals a GNL3L-mediated mechanism that modulates the transcriptional activities of ERR proteins.

Results

GNL3L interacts with ERR family genes

To determine the unique function of GNL3L in vertebrates, we searched for proteins that interact with GNL3L but not with nucleostemin. A yeast two-hybrid approach was employed where full-length GNL3L was fused to a GAL4 DNA binding-domain and used to screen a mouse E17.5 brain cDNA library. From a total of 5 million clones screened, two positive clones were identified. They encoded the same in-frame partial sequence of ERRγ (Clone #43, residues 27–458). The interaction between GNL3L and clone #43 or full-length ERRγ was confirmed by affinity binding assays in which agarose-bound GST fusion proteins of clone #43 and full-length ERRγ, but not the GST backbone protein, were able to retain hemagglutinin (HA)-tagged GNL3L specifically (Fig. S1A in supplemental data). We use coimmunoprecipitation assays to verify the interaction between GNL3L and ERRγ and to determine if nucleostemin or Ngp1 can bind ERRγ. HEK293 cells were cotransfected with Myc-tagged ERRγ and one of the nucleostemin family genes tagged with an HA-epitope. Protein complexes were precipitated with anti-Myc or anti-HA antibody, and immunodetected for the HA- or Myc-tagged proteins (Fig. 1A). We found that only GNL3L, but not nucleostemin or Ngp1, was copurified with ERRγ by anti-Myc immunoprecipitation (row 1). Consistently, ERRγ was detected only in the GNL3L protein complex, but not in the nucleostemin or Ngp1 protein complexes, precipitated by anti-HA antibody (row 3). Like GNL3L, ERRγ belongs to a gene family of three. To determine if GNL3L interacts with only ERRγ or with multiple members of the ERR gene family, HEK293 cells were transfected with HA-tagged GNL3L and Myc-tagged ERRα, ERRβ, or ERRγ expression plasmids. Our results show that GNL3L and all members of the ERR gene family can be copurified in the same protein complexes precipitated by either anti-Myc or anti-HA antibody (Fig. 1B, rows 1 and 3). In contrast, no physical interaction between ERRα (or ERRβ) and nucleostemin (or Ngp1) was detected by affinity-binding assays (Fig. S1B in supplemental data). Based on these results, we conclude that only GNL3L in the nucleostemin family can form stable protein complexes with the ERR family genes.

ERRγ colocalizes with GNL3L in the nucleoplasm

To determine whether the interaction between GNL3L and ERRγ is physiologically possible, we examined the expression patterns of GNL3L, ERRβ, and ERRγ by multi-tissue northern blot analyses. GNL3L was expressed most highly in the neural tissues, including the brain and eye, and was also detected in the muscle and kidney at low levels (Fig. 1C). Parallel blots showed that ERRβ was expressed most abundantly in the kidney, followed by the eye, testis, heart, and muscle. High-level expression of ERRγ was seen in the heart and eye, followed by the brain, kidney, and muscle. To decide where within the cell this interaction might occur, green fluorescent protein (GFP)-tagged GNL3L or ERRγ was expressed in U2OS cells that express both GNL3L and ERRγ endogenously. We found that GNL3L was distributed both in the nucleolus and in the nucleoplasm, whereas ERRγ was localized exclusively in the nucleoplasm (Fig. 1D). These data show that GNL3L correlates better with ERRγ than with ERRβ in their tissue expression patterns. Within the cell, ERRγ and GNL3L are colocalized in the nucleoplasm. In the nucleolus, only GNL3L is found.

The intermediate domain of GNL3L interacts with the AF2 domain of ERRγ

To gain insight into the functional importance of the GNL3L-ERRγ interaction, we first identified the interacting domains of these two proteins using a panel of truncated GNL3L and ERRγ mutants (Fig. 2A, D). To define the ERRγ-binding domain in GNL3L, agarose-bound GST-ERRγ fusion protein was used to pull down the wild-type and mutant GNL3L proteins (Fig. 2B). Our results showed that ERRγ is able to bind GNL3L mutants that are deleted of the BC-domain (dBC) or the G-domain (dG), as well as the N166I mutant that contains an Asn-to-Isl mutation on residue 166 in the G4 domain, which abolishes the GTP-binding capability of GNL3L (unpublished data). Notably, the GST-ERRγ fusion protein failed to retain mutants without the intermediate (I)-domain (dI and G3l-G, Fig. 2B), indicating that the I-domain is necessary for the ERRγ binding of GNL3L. Different GNL3L mutants displayed distinctive subcellular distribution patterns not related to their ERRγ-binding abilities. The dBC and G3l-

G mutants were localized in the nucleoplasm and cytoplasm (Fig. 2C1 and C2). The dG and dI mutants were localized more in the nucleolus than in the nucleoplasm (Fig. 2C3 and C4), whereas the N166I mutant was diffusely distributed in the nucleus (Fig. 2C5). On the ERRγ side, its protein structure consists of the AF1, DBD, LBD, and AF2 domains (Fig. 2D). GST fusion proteins of the full-length ERRγ, the AF1-domain deletion mutant (dAF1), and the LBD deletion mutant with an intact AF2-domain (dLBD) were able to bind the wild-type GNL3L. In contrast, GST fusion proteins of the AF2-domain deletion mutant (dAF2), the last 245 residues containing the LBD and AF2 domains (LBD-AF2), and a mutant deleted of the LBD and AF2 domain (AF1-DBD) were unable to retain GNL3L, demonstrating that the AF2 domain is necessary but not sufficient for the GNL3L binding of ERRγ (Fig. 2E). Based on these results, we conclude that the interaction between GNL3L and ERRγ requires the I-domain of GNL3L and the AF2 domain of ERRγ, and is independent of the GTP binding and nucleolar localization of GNL3L.

Overexpression of a nucleolar form of GNL3L brings ERRβ and ERRγ into the nucleolus

Given that GNL3L, but not ERRγ, is localized in the nucleolus, we test the idea whether coexpression of GNL3L can bring ERRγ into the nucleolus. Using confocal analysis, we determined the distribution of ERRγ when coexpressed with the wild-type GNL3L (WT), a nucleolar form of GNL3L (NoG3l), or an I-domain mutant of GNL3L fused to an SV40 nuclear localization sequence (NLS) (nls-I). NoG3l was created by replacing the BC-domain of GNL3L with the BC-domain of nucleostemin (indicated by the grey bar in Fig. 3A) because nucleostemin has a stronger nucleolar localization capability than GNL3L does and does not bind ERRγ. The I-domain mutant was fused with an SV40 NLS because it lacks endogenous NLS of its own. Affinity-binding assays confirmed that both NoG3l and nls-I mutants were capable of binding ERRγ (Fig. 3B). While ERRγ by itself was distributed outside of the nucleolus (Fig. 3C1 and C1'), overexpression of wild-type GNL3L (Fig. 3C2 and C2') and NoG3l (Fig. 3C3 and C3') increased the ERRγ fluorescence signal in the nucleolar region compared to cells expressing ERRγ by itself. Notably, in cells overexpressing the NoG3l mutant, the ERRγ signal accumulated in the nucleolar region, particularly in the periphery of the nucleolus. In contrast, the nls-I mutant failed to alter the ERRγ distribution (Fig. 3C4), and the distributions of the wild-type GNL3L and NoG3l were unaltered by coexpression of ERRγ (Fig. 3C2 vs. C5, and C3 vs. C6). Overexpression of GNL3L and its mutants exerts the same effects on the distribution of ERRβ. While ERRβ by itself displays a nucleoplasmic distribution (Fig. 3D1 and D1'), both the wild-type GNL3L protein (Fig. 3D2 and D2') and the NoG3l mutant (Fig. 3D3 and D3') are able to increase the nucleolar intensity of ERRβ. By comparison, NoG3L has a stronger effect on bringing ERRβ into the nucleolus than the wild-type GNL3L does. In contrast, overexpression of the wild-type GNL3L or NoG3l has little or no effect on changing the distribution of ERRα (Fig. 3E). These results demonstrate that overexpression of a nucleolar form of GNL3L is able to change the distributions of ERRβ and ERRγ in the living cells, suggesting the possibility that nucleolar sequestration may underlie the regulation of ERRβ and ERRγ by GNL3L.

GNL3L suppresses the transcriptional activities of ERR family genes

To investigate whether GNL3L can modulate the transcriptional activity of the ERR genes, an *in vivo* cell-based luciferase assay system was set up where CV-1 cells were cotransfected with a Firefly luciferase reporter construct driven by three repeats of a consensus palindromic estrogen response element (ERE, see Methods), an ERRγ expression plasmid, and a Renilla-null luciferase reporter construct. The ERE-specific transcriptional activity was determined by the ratio between the Firefly and Renilla luciferase activities in the same sample, which represent the ERE-driven and the basal activities, respectively. This dual luciferase assay system was used to eliminate variations caused by transfection and by non-specific effects on the common transcription-translational machinery. The Firefly-to-Renilla luciferase activity ratio for each experiment was expressed as the fold of increase over the negative sample not transfected with ERRγ. Our results show that ERRγ can increase the ERE-specific transcriptional activity 6 times (6.0±0.5, mean±*s.e.m.*) higher than that of the control sample, and coexpression of GNL3L is able to attenuate the ERRγ-dependent increase by 50 percent (2.9±0.2) (Fig. 4A1, WT). This ERRγ inhibitory effect of GNL3L requires its ERRγ-interacting domain, as samples coexpressing the dI mutant fail to show such a repressive activity. To determine whether this inhibition is caused by nucleolar sequestration of ERRγ by GNL3L, a nucleolar form of GNL3L (NoG3l) or a nucleoplasmic mutant of GNL3L (dBC) was coexpressed with ERRγ. Our results demonstrate that both NoG3l and dBC are able to inhibit the ERRγ-mediated transcriptional activity stronger than or as strong as the wild-type GNL3L. To test whether this transcriptional repressive effect of GNL3L can act on other members of the ERR family, we set up the same transactivational assay for ERRβ and ERRα. Our data show that GNL3L is able to reduce the transcriptional activity of ERRβ from 4.7 (±0.3) times to 2.1 (±0.1) times over the control sample in an I-domain dependent manner, and both NoG3l and dBC inhibit ERRβ as much as the wild-type protein does (Fig. 4B1). Compared to ERRβ and ERRγ, the ERRα-dependent increase of ERE-driven transactivation is less (2.7 ±0.1). Although the wild-type GNL3L and the NoG3 mutant can reduce the ERRα-mediated transcriptional activity, the dBC mutant fails to do so (Fig. 4C1). The GNL3L effect on the transcriptional activities of the ERR family genes is specific, as GNL3L does not suppress the estradiol (E2)-induced ERα-mediated transactivation using the same reporter assay system (p=0.27, Fig. 4D). Finally, we confirm that these different effects of wild-type and mutant GNL3L on the transcriptional activities of ERR genes are not caused by different expression levels of the GNL3L or ERR proteins (Fig. 4A2, B2, C2).

To confirm the inhibitory effect of GNL3L from a loss-of-function angle, a small interference RNA (siRNA) approach was used to knockdown the expression of endogenous GNL3L in HEK293 cells. The knockdown efficiency of the GNL3L-specific siRNA duplex-1 (siGNL3L-1) and duplex-2 (siGNL3L-2) was determined at the protein level in an HEK293 cell line that stably expresses HA-tagged GNL3L, and estimated to be 83% and 84%, compared to the control siRNA (siNEG) knockdown sample (siNEG) (Fig. 5A). In the siNEG-treated HEK293 cells, ERRγ elicited an 11-fold induction on the ERE-driven transcription. In the siGNL3L-1 and siGNL3L-2-treated cells, the ERRγ-mediated transcriptional activities were significantly increased over the 11-fold induction seen in the siNEG-treated sample (25.7±1.3 for siGNL3L-1 and 25.3±1.0 for siGNL3L-2; $p < 0.0001$, n=9) (Fig. 5B). Reducing the amount of GNL3L also increased the ERRβ-and ERRα-mediated transactivation on the ERE-driven promoters (Fig. 5C, D). By comparison, GNL3L-specific siRNA treatment had less effect on the ERRβ- and ERRα-mediated transcription than on the ERRγ-dependent transactivation. The ERRα (or ERRβ)-dependent transcriptional activities are 1.7±0.1 (or 6.8±0.2), 2.4±0.1 (or 10.7 ±0.3), and 2.6±0.1 (or 11.7±0.4) in the siNEG, siGNL3L-1, and siGNL3L-2-treated samples, respectively. Together, our data demonstrate that GNL3L can inhibit the transcriptional functions of the ERR genes without entering the nucleolus.

GNL3L competes with SRC1 and SRC2 (GRIP1) for ERRγ binding

To look for a mechanism other than nucleolar sequestration to explain the GNL3L-mediated inhibition of ERR transactivation, we examine the possibility that GNL3L binding of ERRγ may prevent ERRγ from accessing coactivators such as SRC1 and SRC2 (GRIP1), which have been shown to bind the AF2 domain of ERRγ as well (Hong et al., 1999). To test this idea, GST fusion proteins of ERRγ were used to pull down whole cell lysates containing a fixed amount of SRC1 or SRC2, mixed with increasing amounts of GNL3L (Fig. 6A1 and B1). In each sample, whole cell proteins were adjusted to the same amount. Western analyses of the agarose-bound protein fractions showed that less SRC1 or SRC2 proteins were retained by GST-ERRγ as more GNL3L proteins were bound by ERRγ (Fig. 6A1 and B1, (R)). The ability of GNL3L to compete with SRC1 and SRC2 for ERRγ binding is abolished by a deletion of its ERRγ-interacting I-domain (Fig. 6A2 and B2). Conversely, to determine if SRC1 or SRC2 can displace GNL3L from the ERRγ protein complex, GST-ERRγ fusion proteins were used to pull down a fixed amount of GNL3L in the presence of increasing amounts of SRC1 or SRC2. Our results show that both SRC1 (Fig. 6C) and SRC2 (Fig. 6D) can reduce the amount of GNL3L bound by GST-ERRγ in a dose-dependent manner. These data demonstrate that bindings between ERRγ and GNL3 and between ERRγ and SRC1 or SRC2 are mutually exclusive, and suggest that blocking ERRγ from accessing SRC1 and SRC2 may be responsible for the transcriptional inhibitory activity of GNL3L.

Coexpression of GNL3L increases the mobility and decreases the SRC1 component of the ERRγ DNA-protein complex

To determine whether GNL3L forms a high-order DNA-protein complex with ERRγ, electrophoretic mobility shift assays (EMSA) were conducted using a radiolabeled probe containing a canonical ERE sequence (TCA**GGTCA**CTG**TGACC**TGA) and cell extracts expressing the indicated proteins (Fig. 7A). Compared to the probe alone (lane 1) and vector-transfected cell lysate samples (lane 2), the Myc-tagged ERRγ-transfected sample (lane 3) yields an ERE-ERRγ-specific DNA-protein complex (arrow b). This complex can be competed by excess unlabeled probes (lane 4) and supershifted by anti-Myc antibody (lane 5, arrow a). When ERRγ was coexpressed with GNL3L, two fast-moving DNA-protein complexes were identified (arrows d and e, lane 6), and no additional slow-moving complexes were seen. The mobility of the fast-moving complexes can be retarded by anti-Myc antibody (lane 7, arrow c), but not by anti-HA antibody (lane 8), suggesting that they contain ERRγ but not GNL3L. GNL3L alone fails to interact with the ERE probe (lane 9). The intensity of the fast-moving complex d was reduced when ERRγ was coexpressed with a GNL3L mutant deleted of its ERRγ-binding I-domain (dI), indicating that the appearance of this fast-moving complex depends on the interaction between GNL3L and ERRγ.

The increased mobility of the ERRγ DNA-protein complex by GNL3L may be caused by protein cleavage of ERRγ or by changes in the protein conformation or component of the ERRγ DNA-protein complex; failure of this fast-moving complex d to be supershifted by anti-HA antibody indicates that it does not contain GNL3L or the HA-epitope of GNL3L is masked in this particular protein conformation. To address these different possibilities, we retrieved the fast (complex d) and slow (complex b) moving protein complexes from the EMSA gel and analyzed the protein amount and size of ERRγ, GNL3L, SRC1, and SRC2 in these two complexes by western blottings (Fig. 7B). Anti-Myc western analysis shows that the size of the ERRγ protein remains the same in both complexes, excluding the possibility that the increased mobility is a result of ERRγ protein cleavage. Anti-HA western blotting detects no GNL3L protein in the retrieved protein complexes, consistent with the idea that GNL3L does not bind the DNA-bound ERRγ. Notably, we are able to detect SRC1 in the slow-moving complex b but not in the fast-moving complex d, demonstrating that coexpression of GNL3L reduces SRC1 binding to the ERRγ DNA-protein complex. Although the SRC2 protein is present in both complexes, the amount of SRC2 relative to ERRγ is reduced in the fast-moving complex d compared to the slow-moving complex b, suggesting that SRC2 binding to ERRγ is also diminished by GNL3L coexpression.

GNL3L suppresses the SRC-mediated transcriptional coactivation on ERRγ

Next, we address the issue if GNL3L interferes with the function of SRC proteins as coactivators for ERRγ. In a cell-based reporter system similar to that described in Fig. 4, coexpression of ERRγ and SRC1 is able to produce an 8-fold increase (8.0 ± 0.3) in the ERE-specific transcriptional activity compared to the control sample, which is 1.7 times higher than the sample expressing only ERRγ (4.8 ± 0.3) (Fig. 8A). When coexpressed with GNL3L, the luciferase activity is reduced to 3.6 (± 0.2, for 100ng of GNL3L) and 2.5 (± 0.2, for 200ng of GNL3L) times that of the control sample, which represents a 55 and 70 percent reduction compared to the sample expressing both ERRγ and SRC1. Again, a deletion of the ERRγ-interacting domain (dI) abolishes the suppressive activity of GNL3L (P value = 0.17). The ability of GNL3L to inhibit the transcriptional coactivator activity can also work on SRC2 (Fig. 8B). Here we show that GNL3L, but not the dI mutant, can reduce the ERRγ-SRC2-mediated 7.4-fold increase (7.4 ± 0.2) in the luciferase activity down to 3.4 (± 0.2, for 100ng of GNL3L) and 2.1 (± 0.2, for 200ng of GNL3L) folds compared to the control sample. The dI mutant fails to exhibit this inhibitory activity (P value = 0.58). These data demonstrate that GNL3L can suppress the SRC1- and SRC2-mediated coactivation of ERRγ in an I-domain dependent manner.

Discussion

In this manuscript, we identify a GNL3L-mediated mechanism that suppresses the transcriptional activity of ERR family genes by coactivator competition (Fig. 9). We show that ERR binding is a specific property of GNL3L, but not that of nucleostemin or Ngp1. GNL3L and ERRγ colocalize in the nucleoplasm, and their interaction requires the I-domain of GNL3L and the AF2-domain of ERRγ. Gain- and loss-of-function studies reveal that GNL3L possesses the ability to suppress the transcriptional activity of ERR genes, which does not require the nucleolar localization of GNL3L. We also demonstrate that GNL3L can compete with SRC1 and SRC2 for their ERRγ binding, resulting in an increased electrophoretic mobility of the DNA-bound ERRγ complex and an inhibition of the SRC1 and SRC2 coactivator function on ERRγ. The AF2-domain binding, SRC competition, and transcriptional inhibition activities suggest that GNL3L might represent a new class of transcriptional corepressor for nuclear receptors. However, GNL3L fails to form a stable complex with the DNA-bound ERRγ, and the I-domain of GNL3L, which is necessary and sufficient for ERRγ binding, lacks the LXXLL motif found in most transcriptional coactivators and corepressors that interact with the AF2-domain (Hentschke et al., 2002;Huss et al., 2002;Lee et al., 1998;Rosenfeld and Glass, 2001;Webb et al., 2000;Zhang et al., 2000). These data support the role of GNL3L as a novel regulator for the ERR family genes and argue against its role as a classical transcriptional corepressor. It is worth noting that the absence of interaction between GNL3L and the DNA-bound ERRγ *in vitro* does not exclude the possibility that these two proteins may still coexist in the same DNA-bound complex in the native chromatinized context, as transcription factor binding to the core response element *in vivo* are aided by a number of cofactors as well as by chromatin-remodeling histone proteins. On the other hand, the ability of GNL3L to compete for SRC binding and inhibit the transcriptional function of ERRγ may depend on specific chromatin structures and involve cofactors other than SRC proteins.

GNL3L and ERR proteins are colocalized in the nucleoplasm, but only GNL3L can be found in the nucleolus. The nucleolar localization of GNL3L may create two potential mechanisms that affect its activity in the nucleoplasm. First, GNL3L can enter or exit the nucleolus by itself. In this case, signals that promote the nucleolar accumulation of GNL3L may cause a disinhibition of the ERR activities, and signals that release the nucleolus-bound GNL3L into the nucleoplasm may allow more GNL3L to bind ERR proteins. Alternatively, GNL3L may carry some ERR proteins with it when entering the nucleolus, in which case nucleolar sequestration of ERR proteins may account for some of the inhibitory activity of GNL3L. Although overexpression of a nucleolar form of GNL3L and, to a less extent, the wild-type GNL3L does increase the nucleolar intensity of ERRβ and ERRγ, the non-nucleolar dBC mutant is able to suppress the ERRβ and ERRγ activities as strong as the wild-type GNL3L does. These results demonstrate that the suppressive effect of GNL3L on the ERR activity is not mediated by a nucleolar sequestration mechanism, and that under physiological conditions, the ERR-binding and transcriptional inhibition events of GNL3L take place in the nucleoplasm.

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Nevertheless, the fact that overexpression of NoG3l can bring ERRβ and ERRγ into the nucleolus supports the notion that GNL3L is able to interact with these two proteins *in vivo*.

Unlike ERα whose activity is controlled by hormone binding, no ligand has been identified for the ERR family genes and their transactivation works in a ligand-independent manner. This GNL3L-mediated inhibition on the activities of ERRs provides one mechanism to regulate their functions in a cell context-dependent and dynamic way. At the transcriptional level, although the relative abundance of GNL3L matches that of ERRγ in most tissues we examined, they are distinctively different in the brain and heart. GNL3L is expressed highly in the brain but little in the heart, whereas ERRγ is found at a high level in the heart but not in the brain. The differences between the expression levels of GNL3L and ERRγ in those organs indicate that tissues expressing the same level of ERRγ may exhibit differential ERR activities depending on their GNL3L expression. In the adult brain where little ERRγ is found, GNL3L may have other regulatory targets. At the post-translational level, GNL3L is partitioned between the nucleolus and the nucleoplasm by a dynamic process (Meng and Tsai, unpublished data). Like nucleostemin (Meng et al., 2006; Tsai and McKay, 2005), the nucleolar accumulation of GNL3L is controlled by its GTP binding and a N-terminal basic domain (Rao et al., 2006) (Meng and Tsai, unpublished data). Notably, the nucleolar residence of GNL3L is significantly shorter than that of nucleostemin (Meng and Tsai, unpublished data). The transient residence of GNL3L in the nucleolus may explain why nucleolar compartmentalization of GNL3L does not seem to play a role in its ability to suppress the ERR transcriptional function.

In conclusion, it is known that the ERR family genes are transcriptionally active without the ligand, but it is unclear if and how their activities can be controlled in a dynamic manner. Our work unravels a GNL3L-mediated mechanism that modulates the transcriptional activity of ERRγ by coactivator competition. Given the important role of the ERR genes in embryogenesis and tumorigenesis, the differential regulation of their activities by GNL3L can provide us with new insight into these two processes in a cell type-specific manner.

Materials and Methods

Recombinant plasmids and mutation analyses

Full-length ERR family genes were cloned from mouse brain cDNAs by reverse transcription-PCR. Deletions and point mutations of GNL3L and ERRγ were introduced by the stitching PCR method as described previously (Tsai and McKay, 2002; Tsai and McKay, 2005). The final PCR products were subcloned into pCIS expression vectors and confirmed by sequencing.

Cell culture, transfection, siRNA knockdown, and immunostaining

We used HEK293 cells for biochemical studies because of their high transfection efficiency and protein production and U2OS cells for distribution analyses. Cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 5% fetal bovine serum (Hyclone), penicillin (50 IU/ml), streptomycin (50ug/ml), and glutamine (1%). Plasmid transfections were performed using a standard calcium phosphate method for HEK293 cells or the Lipofectamine-Plus reagent (Invitrogen) for U2OS cells. Immunofluorescence studies were performed one day after transfection as described previously (Tsai and McKay, 2005). For siRNA knockdown experiments, cells were transfected with siRNA duplex (20uM) for 12–24 hours using the Oligofectamine reagent (Invitrogen) and analyzed 2 days later. Targeted sequences for siRNA duplexes are as followed: siGNL3L-1: 5’-AAA AAC GCA GGA CCA UUG AGA-3’; siGNL3L-2: 5’-AAC UAU UGC CGC CUU GGU GAA-3’; siNEG: 5’-AAU GAC GAU CAG AAU GCG ACU-3’.

Primary antibodies include: monoclonal anti-HA antibody (1:2000X; HA.11, Covance), monoclonal anti-Myc antibody (1000X, Covance), and monoclonal anti-fibrillarin antibody (1000X, EnCor). Secondary antibodies are conjugated with Rodamine-X or FITC.

Yeast two-hybrid screen

Full-length mouse *GNL3L* was subcloned in the pAS2-1 vector and used as a bait to screen an adult mouse brain cDNA library in the pACT2 vector (Clontech). The bait and library plasmids were cotransformed into *Saccharomyces cerevisiae* strain Y190 and selected for both $histidine^+$ and β-$galactosidase^+$ phenotypes. cDNA plasmids were re-transformed into *Escherichia coli* HB101 by electroporation and expanded for further analysis.

Coimmunoprecipitation

Cells were harvested in NTEN buffer (20 mM Tris pH8.0, 150 mM NaCl, 1 mM EDTA, 0.5% NP40, 0.1 mM DTT, supplemented with 1 mM PMSF, 1 ug/ml leupeptin, 0.5 ug/ml aprotinin, 0.7 ug/ml pepstatin A, and 1 uM E64). Lysates were incubated with monoclonal anti-HA (HA. 11, Covance), monoclonal anti-Myc (9E10, Covance), or mouse IgG for 1 hour, followed by incubation with protein G sepharose beads (Pharmacia) for an additional 4 hours at 4C. Immunoprecipitates were washed 5 times with RIPA buffer (1X PBS, 0.1% SDS, 0.5% sodium deoxycholate, 1% NP40, 1 mM PMSF, 1 ug/ml leupeptin, 0.5 ug/ml aprotinin, 0.7 ug/ml pepstatin A, and 1 uM E64), fractionated by 10% sodium dodecyl sulfatepolyacrylamide gel electrophoresis (SDS-PAGE), and transferred to Hybond-P membranes (Amersham). Specific signals were detected by Western blotting with polyclonal anti-HA or anti-Myc antibodies and horseradish peroxidase-conjugated secondary antibody.

Affinity binding and competition assays

Full-length cDNAs of *GNL3L* and ERRγ were subcloned into the pGEX4T-2 vector. GST fusion proteins were expressed in BL21/DE3 as described previously (Tsai and McKay, 2002; Tsai and Reed, 1997). Epitope-tagged proteins were expressed in HEK293 cells and extracted in phosphate-buffered saline (PBS)-Triton X-100 (1%) buffer, supplemented with protease inhibitors. Sepharose-bound GST fusion proteins (2–5ug) were incubated with whole cell lysates for 2 hours at 4C, washed five times with extraction buffer, including two times of high-salt buffer (extraction buffer plus 500mM of NaCl), fractionated on 10% SDS-PAGE, and detected by Western blot analyses.

Northern blot analyses

Ten micrograms of total RNAs were isolated from CD-1 mice using Trizol solutions (Invitrogen), fractionated on 1% formamide denaturing agarose gels, and transferred onto Hybond XL membrane (Amersham). Filters were then hybridized with α-^{32}P-labeled probes at 65C overnight and washed with high stringency. Plaque date was counted as embryonic day 0.5 (E0.5).

Image acquisition

Confocal images were captured on a Zeiss LSM510 confocal microscope using a 63X plan-apochromat oil objective. Images were scanned using the multi-track program, a 512×512 frame size, 3X zoom, and <1.4um optical thickness. Detector gain and amplifier offset were adjusted to ensure that all signals were appropriately displayed within the linear range. Fluorescence intensities were digitally quantified in Fig. 3C', D' and E' using the profile display mode along the path indicated by arrow.

Dual luciferase assays

For gain-of-function experiments, CV1 cells were grown in DMEM supplemented with 5% charcoal/dextran-treated FBS. Transient transfection was performed in 24-well plates using the Lipofectamine-Plus reagent. Total DNA amount in each well was adjusted to 2ug using the empty expression vector. Cell extracts were prepared 30 hours after transfection. HEK293 cells were used for siRNA knockdown experiments because of their high GNL3L expression level. HEK293 cells were split and grown in DMEM supplemented with 5% chacoal/dextran-treated FBS. On the next day, transfections of siRNA duplexes were performed in 24-well plates using the Oligofectamine reagent (Invitrogen). Firefly (100ng) and Renilla (10ng) reporter plasmids, ERR (50ng), ERα (100ng), GNL3L-related genes (200ng or as specified), and/or SRC (50ng) expression vectors were transfected on the 4th day, and cell extracts were prepared one day later. For E2 stimulation (Fig. 4D), cells were treated with 100nM of E2. Firefly and Renilla luciferase activities were measured by the Dual-Luciferase Reporter Assay System (Promega). The expression of the Firefly luciferase reporter gene was driven by three repeats of a synthetic consensus palindromic estrogen response element (ERE, **GGTCA**CTG**TGACC**).

EMSA and post-EMSA western blot

Electrophoretic mobility shift assays (EMSA) were carried out as described previously (Tsai and Reed, 1997; Tsai and Reed, 1998) with the following modifications. Recombinant proteins were expressed in HEK293 cells. Whole cell lysates were extracted in buffer containing 40mM HEPES-KOH (pH 7.9), 0.4M KCl, 1mM DTT, 10% glycerol, 0.1mM PMSF, and complete protease inhibitor cocktail (Roche) mixed with specified amounts of probes in 20ul binding reactions, and incubated on ice for 20 minutes. The binding-reaction mixture contains 10mM HEPES (pH7.9), 70mM KCl, 2.5mM $MgCl_2$, 1mM EDTA, 1mM dithiothreitol, 4% glycerol, 20ug/ml salmon sperm DNA, and 200ug/ml poly deoxyinosinic-deoxycytidylic acid. The reaction products were subjected to electrophoresis on a 4% polyacrylamide gel (29:1) in 0.5X Tris-borate-EDTA (TBE) buffer at 4C, and detected by autoradiography. To generate EMSA probes, RT1006 primer was radiolabeled with γ-P^{32} ATP in a T4 kinase reaction, annealed with excess amounts of RT1007 primer, and purified using a QIAquick nucleotide removal kit (Qiagen). RT1006: 5'-GAT CTC TTT GAT CAG GTC ACT GTG ACC TGA CTT TG-3'; RT1007: 5'-GAT CCA AAG TCA GGT CAC AGT GAC CTG ATC AAA GA-3. To determine the protein components in the shifted complexes, the fast and slow mobility complexes were identified by autoradiography, retrieved, and subjected to SDS-PAGE. Western analyses were performed using the mouse anti-Myc, rabbit anti-HA, rabbit anti-SRC1 (abcam, ab2859, 500X), and mouse anti-SRC2 (BD Transduction Laboratories, clone 29, 250X) antibodies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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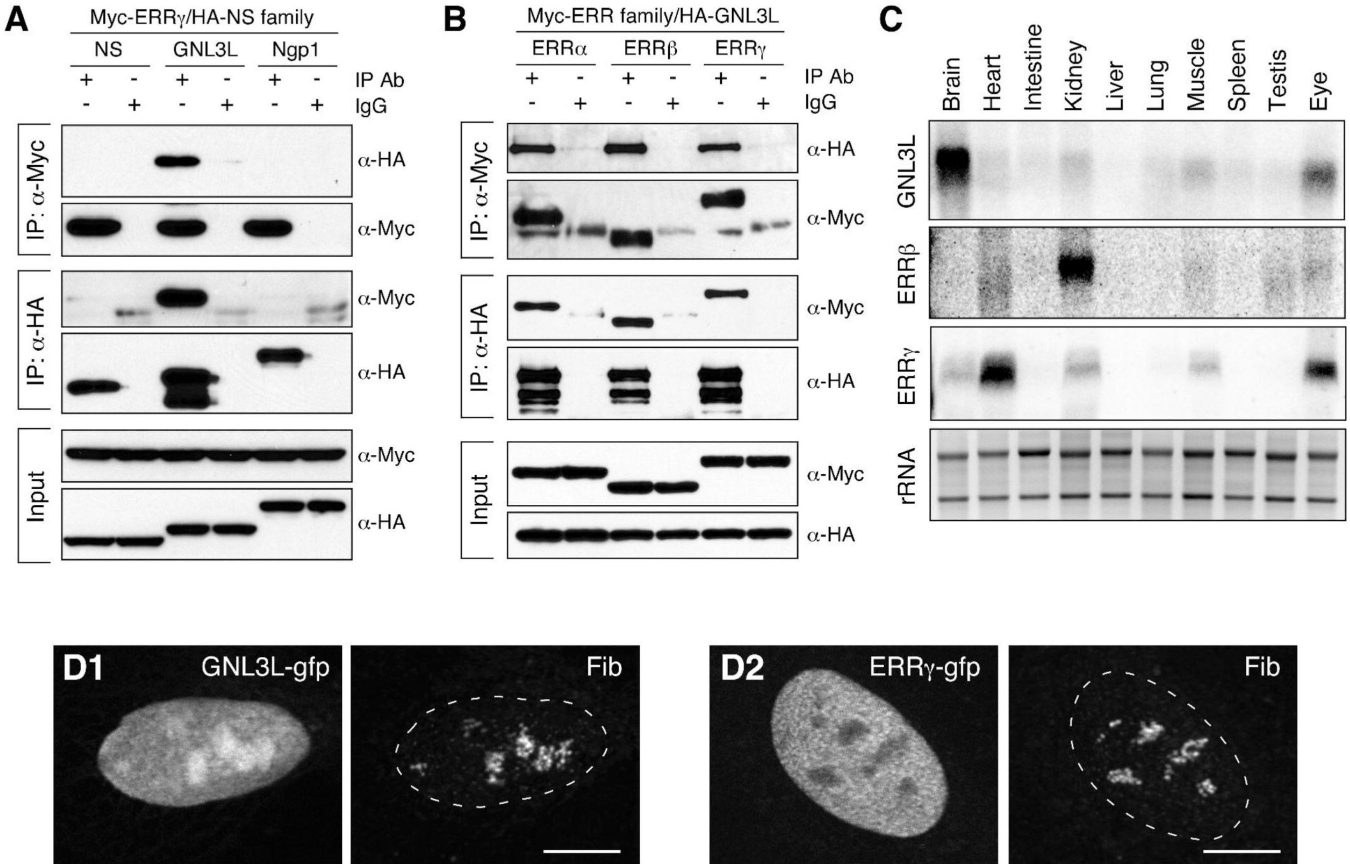


Figure 1. GNL3L interacts with estrogen receptor-related proteins (ERR)-α, β, and γ

Protein interactions between ERRγ and nucleostemin family genes (**A**) and between GNL3L and ERR family genes (**B**) were examined by *in vivo* coimmunoprecipitation assays. HEK293 cells were cotransfected with: (**A**) Myc-tagged ERRγ and HA-tagged nucleostemin, GNL3L, or Ngp1 expression plasmids, or (**B**) HA-tagged GNL3L and Myc-tagged ERRα, β, or γ expression plasmids. Lysates were immunoprecipitated (IP) with anti-Myc antibody (rows 1 and 2, α-Myc) or anti-HA antibody (rows 3 and 4, α-HA). The copurified proteins (rows 1 and 3) and the immunoprecipitates (rows 2 and 4) were immunodetected with the antibodies indicated on the right. Our results show that ERRγ interacts with only GNL3L, but not with nucleostemin or Ngp1, and that GNL3L binds all ERR family proteins. (**C**) Tissue distributions of GNL3L, ERRβ, and ERRγ in adult mice are shown by multi-tissue northern blots. The GNL3L message is expressed primarily in the neural tissues, including the brain and eye, and at a lower level in the kidney and muscle. The expression levels of GNL3L and ERRγ match in the kidney, muscle, and eye, but differ in the brain and heart. (**D**) In U2OS cells, the intensity of the green fluorescent protein (GFP)-tagged GNL3L (GNL3L-gfp) is higher in the nucleolus than in the nucleoplasm. GFP-tagged ERRγ (ERRγ-gfp) is localized exclusively in the nucleoplasm. The nucleolar regions are labeled by anti-fibrillarin (Fib) immunofluorescence in the right panels. Dashed lines demarcate the nucleocytoplasmic boundaries. Bars: 10 um.

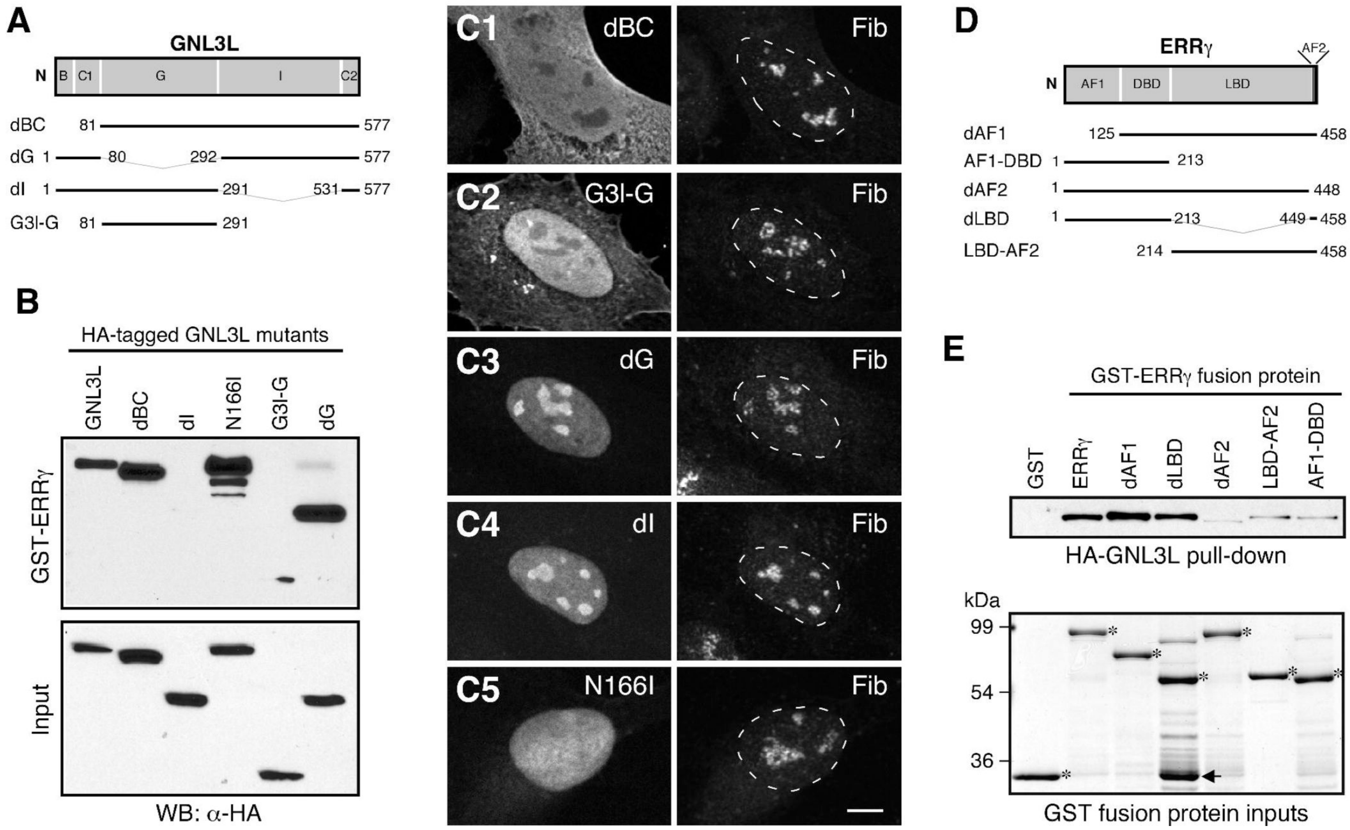
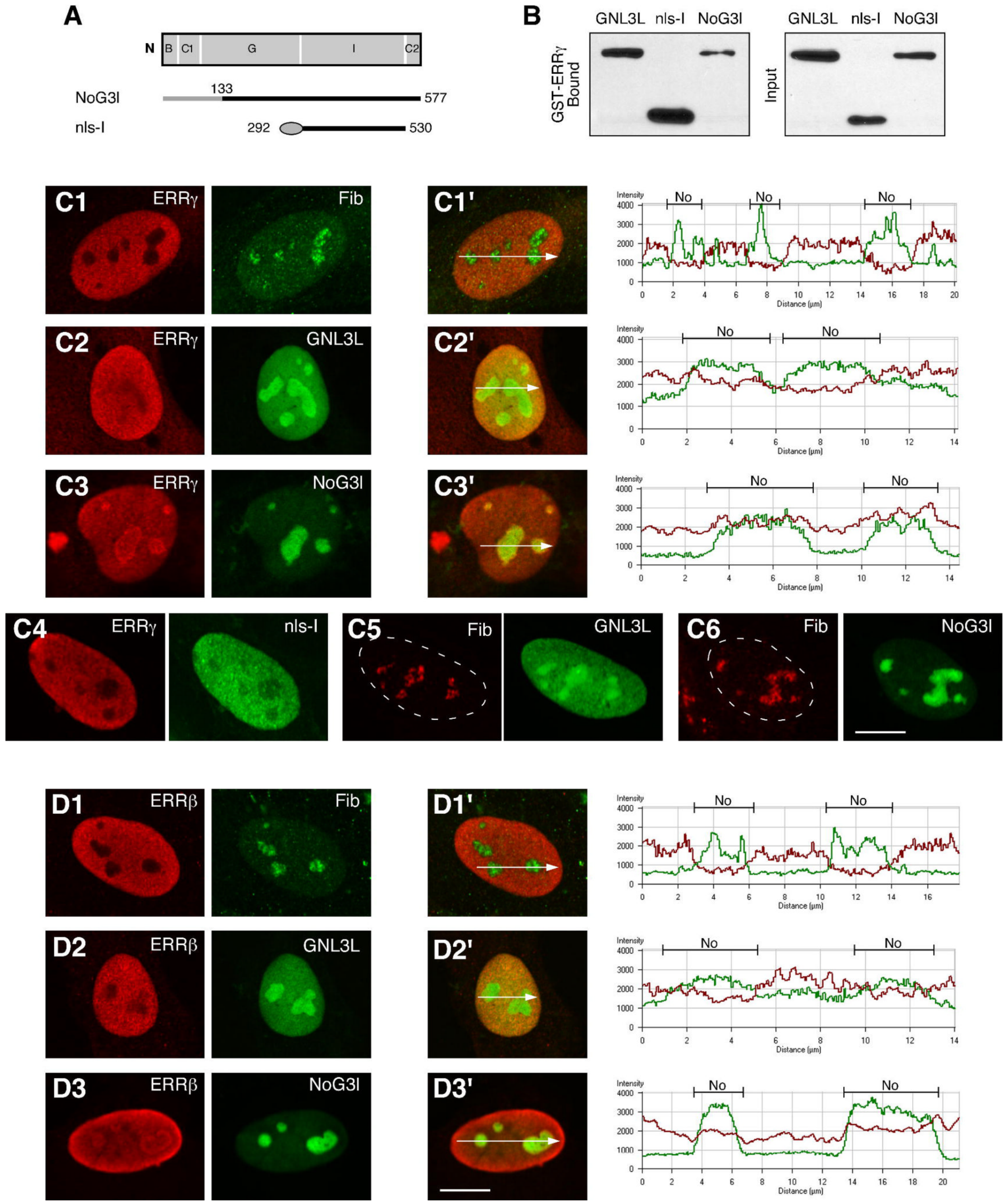


Figure 2. Binding between GNL3L and ERRγ requires the intermediate (I)-domain of GNL3L and the AF2-domain of ERRγ

(**A**) Truncated mutants of GNL3L were used to determine its interacting domain with ERRγ. B, basic; C1 and C2, coiled-coil domain-1 and 2; G, GTP-binding domain; I, intermediate domain. Numbers indicate amino acid positions. (**B**) GST-ERRγ fusion proteins fail to bind mutants that lack the I-domain (dI and G3l-G), but can retain the dBC and the non-GTP-binding mutants, N166I and dG. (**C**) The subcellular distributions of HA-tagged dBC, G3l-G, dG, dI, and N166I mutants were shown by confocal analyses double-labeled with anti-HA (left panels) and anti-fibrillarin (Fib, right panels) antibodies. Bar: 10um. (**D**) Truncated mutants of ERRγ were used to determine its interacting domain with GNL3L. AF1 and AF2, activation function 1 and 2; DBD, DNA-binding domain; LBD, ligand-binding domain. (**E**) Affinity binding assays show that GST fusion proteins of the wild-type ERRγ, the dAF1 mutant, and the dLBD mutant can bind GNL3L, but GST fusion proteins of the dAF2, LBD-AF2, and AF1-DBD mutants cannot (top panel). The amounts of GST fusion proteins used in each reaction, marked by asterisks, are shown in the bottom panel by Commassie blue staining. Some degradation occurs at the fusion site of the GST-dLBD protein (arrow).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C1
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I
C2
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GST-ERRγ Bound
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C1
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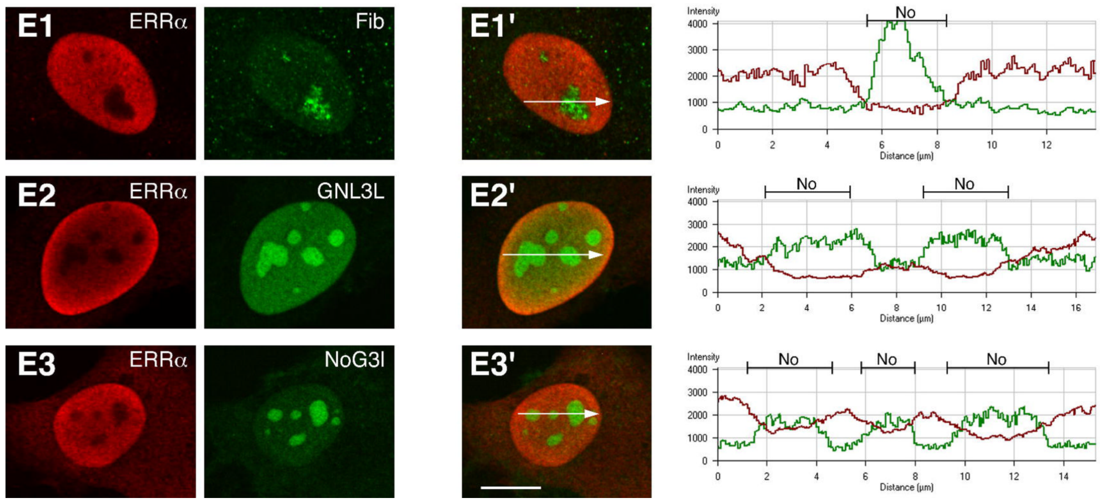

Figure 3. Overexpression of GNL3L brings ERRβ and ERRγ into the nucleolus

(**A**) To generate a nucleolar form of GNL3L (NoG3l), we replaced the N-terminal nucleolus-targeting domain of GNL3L with the corresponding region of nucleostemin (grey bar), which has a stronger nucleolus-targeting activity than GNL3L but lacks the ability to bind ERRγ. To create a nucleoplasmic form of GNL3L (nls-I), we fused the I-domain of GNL3L with an SV40 nuclear localization signal (oval). (**B**) Affinity binding assays show that both nls-I and NoG3l maintain the ability to bind ERRγ. To measure the effect of GNL3L overexpression on the distribution of ERRγ, U2OS cells were transfected with Myc-tagged ERRγ alone (**C1**), Myc-tagged ERRγ and HA-tagged wild-type or mutant GNL3L (**C2–4**), or HA-tagged GNL3L constructs alone (**C5–6**). Double-transfected cells were labeled with anti-Myc (*red*) and anti-HA (*green*) immunofluorescence, and visualized by confocal analyses. Single-transfected cells were immunostained with anti-fibrillarin antibody (Fib) and anti-Myc or anti-HA antibody. The ERRγ (*red*) fluorescence intensities are measured quantitatively along the lines indicated by arrows, shown in the right panels of (**C1'–C3'**), and the nucleolar regions (No) are indicated by the increase of green fluorescence. Compared to cells transfected with only ERRγ, the fluorescence intensity of ERRγ in the nucleolus is increased in cells cotransfected with NoG3l or the wild-type GNL3L. In contrast, the nls-I mutant does not change the distribution of ERRγ (**C4**). Neither does ERRγ alter the distribution of GNL3L (**C5**) or NoG3l (**C6**). The same analyses were performed using ERRβ (**D1–D3**) and ERRα (**E1–E3**). Our results showed that when coexpressed with wild-type GNL3L (**D2**) or NoG3l (**D3**), the ERRβ signals begin to accumulate in the nucleolus. GNL3L overexpression has little or no effect on the distribution of ERRα (**E2–E3**). Bars: 10um.

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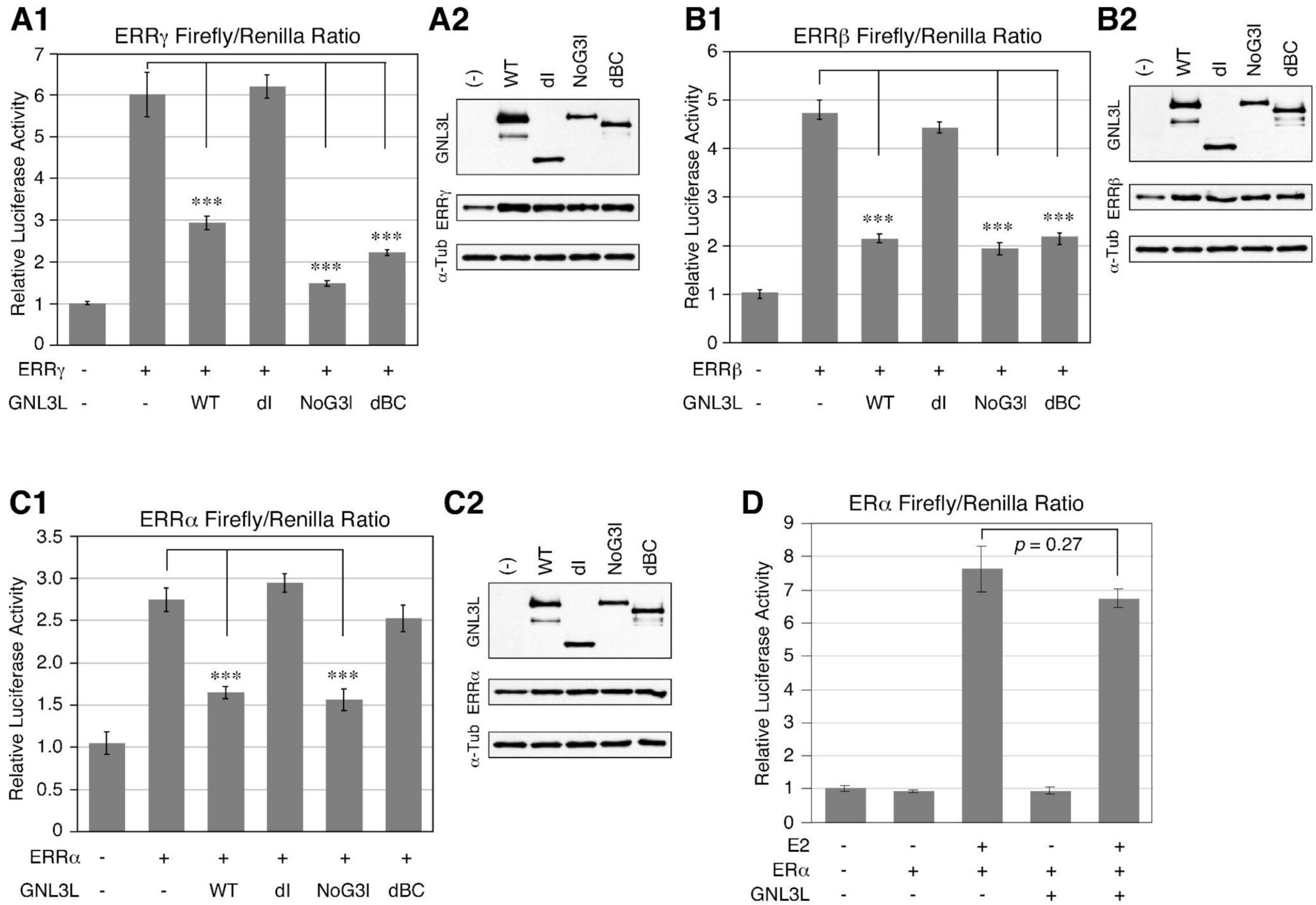

Figure 4. Overexpression of GNL3L inhibits the transcriptional activities of ERR proteins independent of nucleolar distribution

(**A1**) Estrogen response element (ERE)-specific transcriptional activities were measured in CV-1 cells by the ratio between the ERE-driven Firefly luciferase activity and the Renilla-null luciferase activity. ERRγ elicits a six-fold increase in the ERE-specific transcriptional activity. Coexpression of wild-type GNL3L (WT) leads to a 50% reduction in the ERRγ-mediated transcriptional activity. This decrease is reversed by a deletion of the ERRγ-binding I-domain of GNL3L (dI). Coexpression of either the nucleolar form (NoG3l) or the nucleoplasmic form (dBC) of GNL3L suppresses the ERRγ transcriptional activity more than or to the same extent as the wild-type GNL3L protein. Using the same approach, we show that this inhibitory activity of GNL3L can also work on ERRβ (**B1**) and ERRα (**C1**) with the exception that the dBC mutant has little effect on the ERRα-mediated transactivation. Error bars represent stand error of mean (*s.e.m.*). ***, P value < 0.0001. (**A2**, **B2**, **C2**) The expression levels of wild-type and mutant GNL3L proteins and ERR proteins in the experimental samples are compared side-by-side by anti-HA and anti-Myc western blots, respectively. Anti-α-tubulin western blots (α-Tub) are used as loading controls. (**D**) GNL3L fails to suppress the estradiol (E2)-induced transcriptional activity of ERα on the ERE-driven promoter in the same cell-based reporter system.

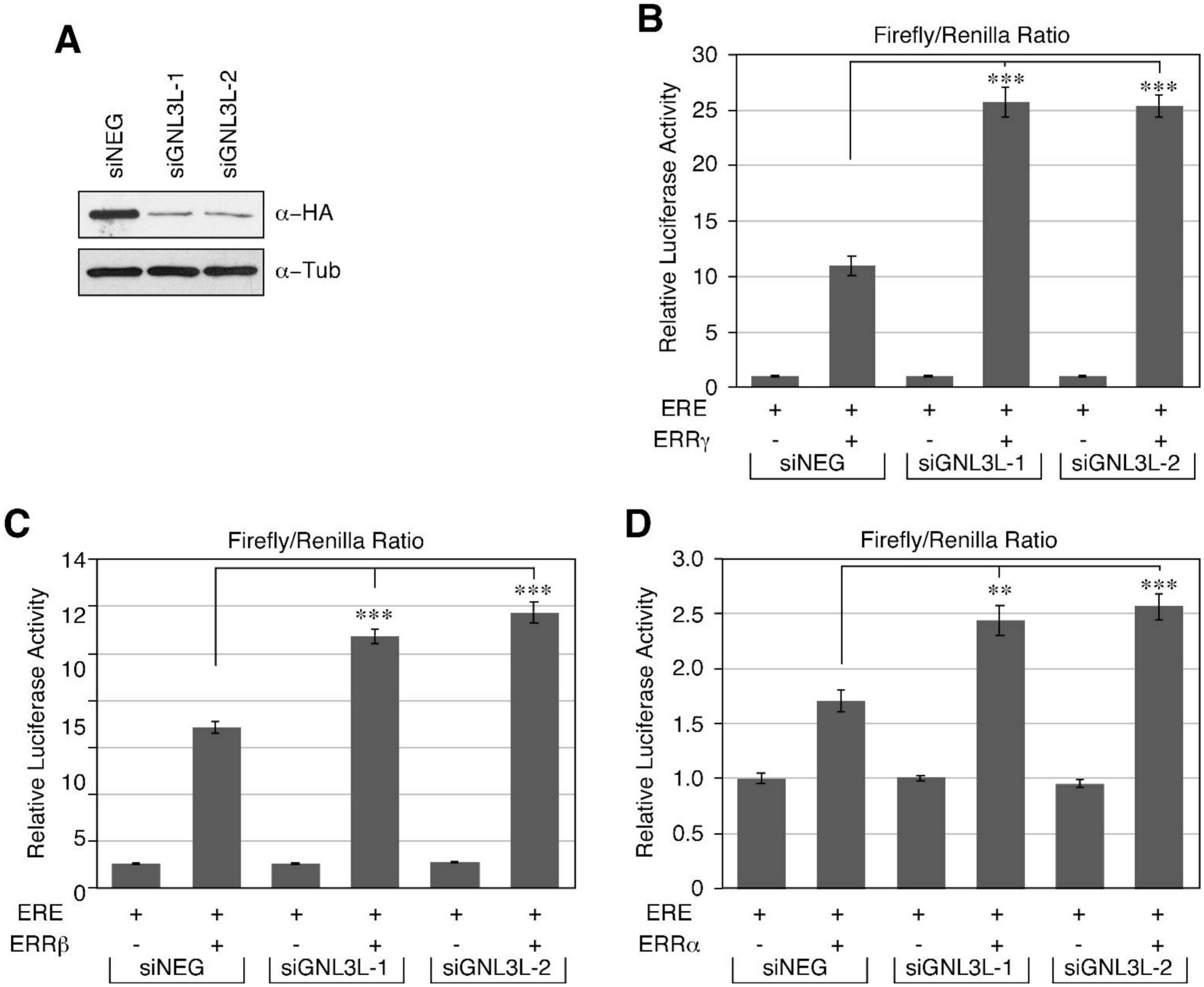

Figure 5. The endogenous GNL3L suppresses the transcriptional activities of ERR family genes

(**A**) To confirm the GNL3L-mediated negative regulation of ERR activities from a loss-of-function angle, a short interference RNA (siRNA) approach was used to knock down the endogenous expression of GNL3L. Compared to the control knockdown sample (siNEG), the protein knockdown efficiencies of GNL3L-specific siRNA duplexes, si*GNL3L*-1 and si*GNL3L*-2, in HEK293 cells stably expressing HA-tagged GNL3L are estimated to be 83% and 84%, respectively. (**B**) Consistent with our overexpression data, the transcriptional activity of ERRγ is increased 2.5 times by the siGNL3L-1 and siGNL3L-2 treatments as compared to the siNEG-treated sample. (**C**, **D**) GNL3L knockdown has the same effect on the ERRβ and ERRα-mediated transactivation, although their increases are less dramatic than the increase in the ERRγ-mediated transactivation. Error bars represent standard error of mean (*s.e.m.*). **, P value < 0.001; ***, P value < 0.0001.

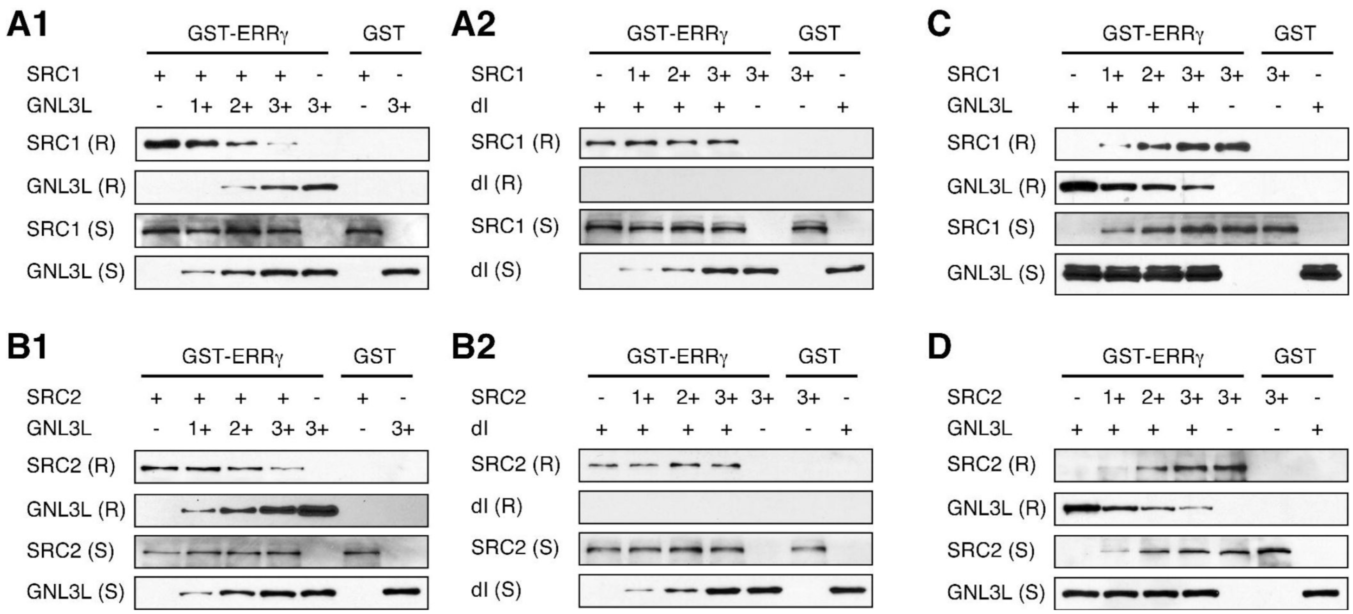

Figure 6. GNL3L competes with SRC1 and SRC2 for ERRγ binding

Agarose-bound GST fusion proteins of ERRγ (1ug) were used to pull down whole cell lysates containing a fixed amount of SRC1 (**A**) or SRC2 (**B**), mixed with increasing amounts of the wild-type GNL3L (**A1**, **B1**) or the dI mutant lacking the ERRγ-interacting domain (**A2**, **B2**). Whole cell proteins in each sample were adjusted to the same amount. In the agarose-retained portions (R), the interaction between GNL3L and ERRγ can reduce the amount of SRC1 and SRC2 bound by ERRγ in a dose-dependent manner, but the dI mutant fails to do so. Conversely, when GST-ERRγ fusion proteins were used to pull down the same amount of GNL3L in the presence of increasing amounts of SRC1 (**C**) or SRC2 (**D**), SRC1 and SRC2 were able to reduce the amount of GNL3L bound by ERRγ in a dose-dependent way as well. Proteins in the agarose-bound fraction and in the supernatant are indicated by (R) and (S), respectively.

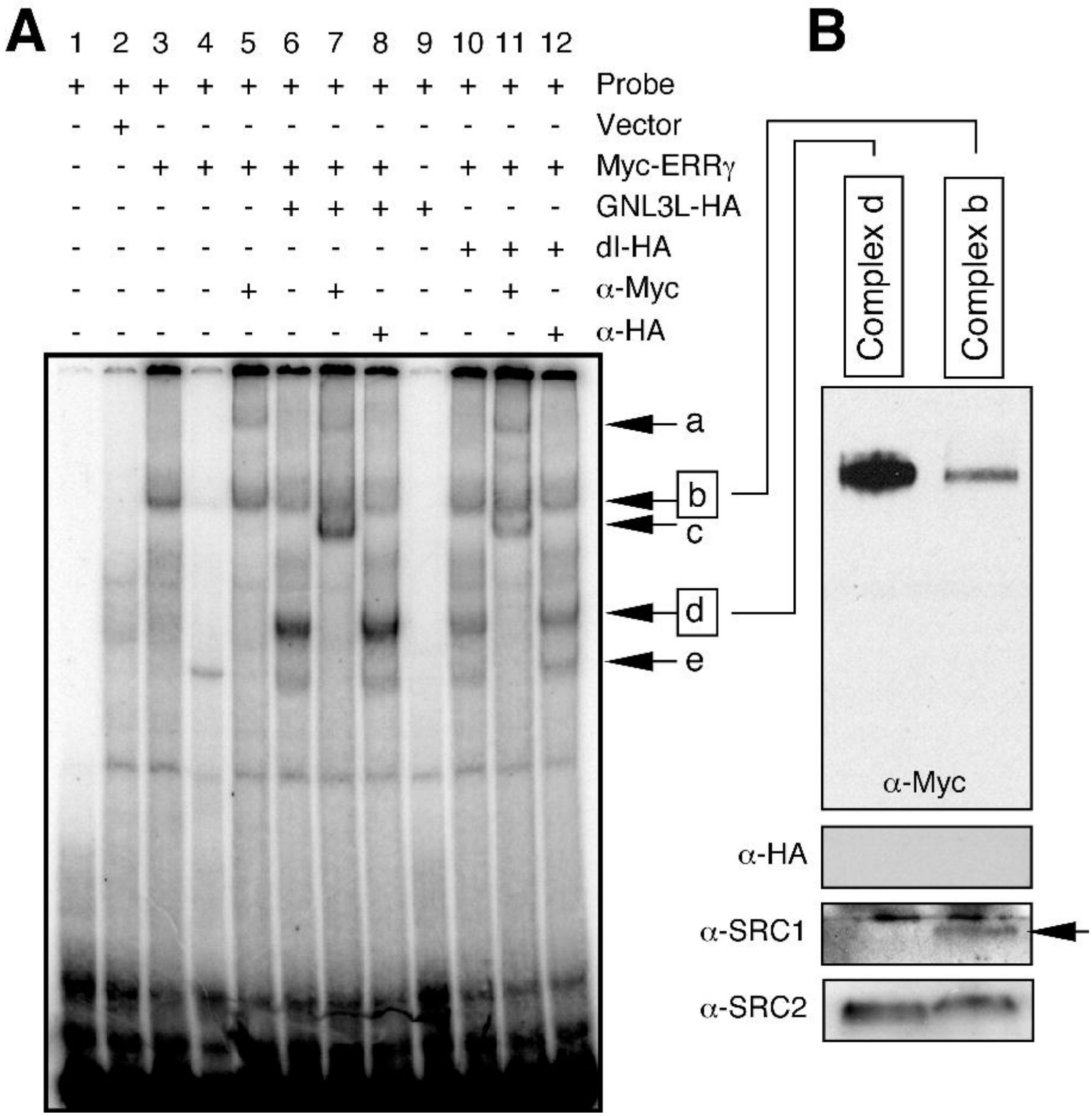

Figure 7. Coexpression of GNL3L increases the electrophoretic mobility of the DNA-bound ERRγ protein complex and reduces its binding with SRC1 and SRC2

(**A**) The GNL3L effect on the DNA binding of ERRγ was examined by electrophoretic mobility shift assays (EMSA) using ERE-containing probes and whole cell lysates expressing the indicated recombinant proteins. Compared to the probe alone (lane 1) and the vector-transfected control sample (lane 2), the ERRγ-specific DNA-protein complex can be identified in lane 3 (arrow b), competed by excess non-labeled probes (lane 4), and supershifted by anti-Myc antibody (lane 5, arrow a). Coexpression of GNL3L produces fast-moving complexes (lane 6, arrows d and e), which can be supershifted by anti-Myc antibody (lane 7, arrow c) but not by anti-HA antibody (lane 8). GNL3L itself cannot bind the ERE probe (lane 9). The intensity of the fast-moving complex d is reduced by a deletion of the ERRγ-binding I-domain of GNL3L (lanes 10–12). (**B**) The fast-moving complex d and the slow-moving complex b were retrieved from the EMSA gel, fractionated in SDS-denaturing PAGE, and analyzed for their ERRγ (α-Myc), GNL3L (α-HA), SRC1, and SRC2 protein components by western blottings. Our results indicate that the increase in the electrophoretic mobility of the ERRγ-DNA complex by GNL3L coexpression can be explained by a loss of SRC1 binding (arrow) and diminished SRC2 binding, rather than by protein cleavage of ERRγ.

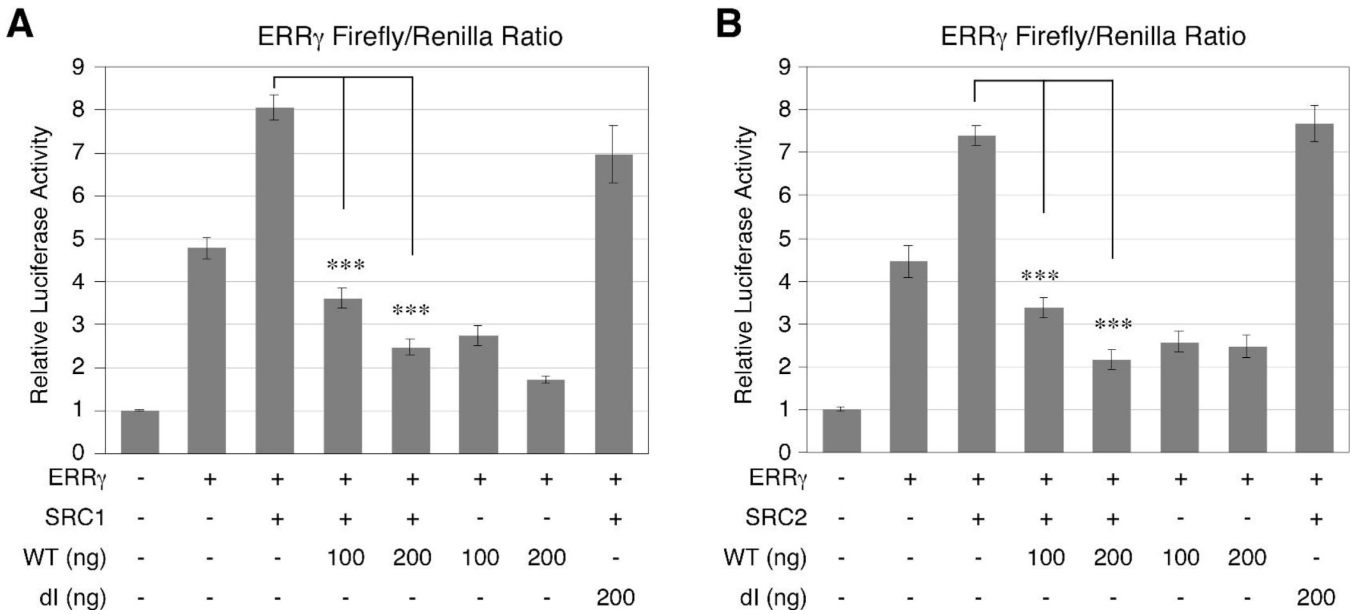

Figure 8. GNL3L suppresses SRC-mediated transcriptional coactivation of ERRγ

(**A**) Using the same cell-based reporter system as described in Fig. 4, we show that the ERE-specific transcriptional activity in cells coexpressing ERRγ and SRC1 (8.0±0.3) is 1.7 times higher than that of the ERRγ-expressing sample (4.8±0.3). When coexpressed with the wild-type GNL3L (WT), this ERRγ and SRC1-mediated ERE-specific transcriptional activity is reduced by 55 and 70 percent compared to the sample expressing both ERRγ and SRC1 in a dose-dependent manner. This inhibitory effect of GNL3L on the SRC1-mediated coactivation of ERRγ requires the I-domain of GNL3L, as a deletion of this domain (dI) fails to suppress the transcriptional activity of ERRγ and SRC1 (P value = 0.17). (**B**) Using the same approach, we show that GNL3L can also suppress the coactivator function of SRC2 on the ERRγ-dependent transcriptional activity in a dose-dependent (54% reduction for 100ng of GNL3L and 71% reduction for 200ng of GNL3L) and I-domain-dependent (P value = 0.58) manner. Error bars represent stand error of mean (*s.e.m.*). ***, P value < 0.0001.

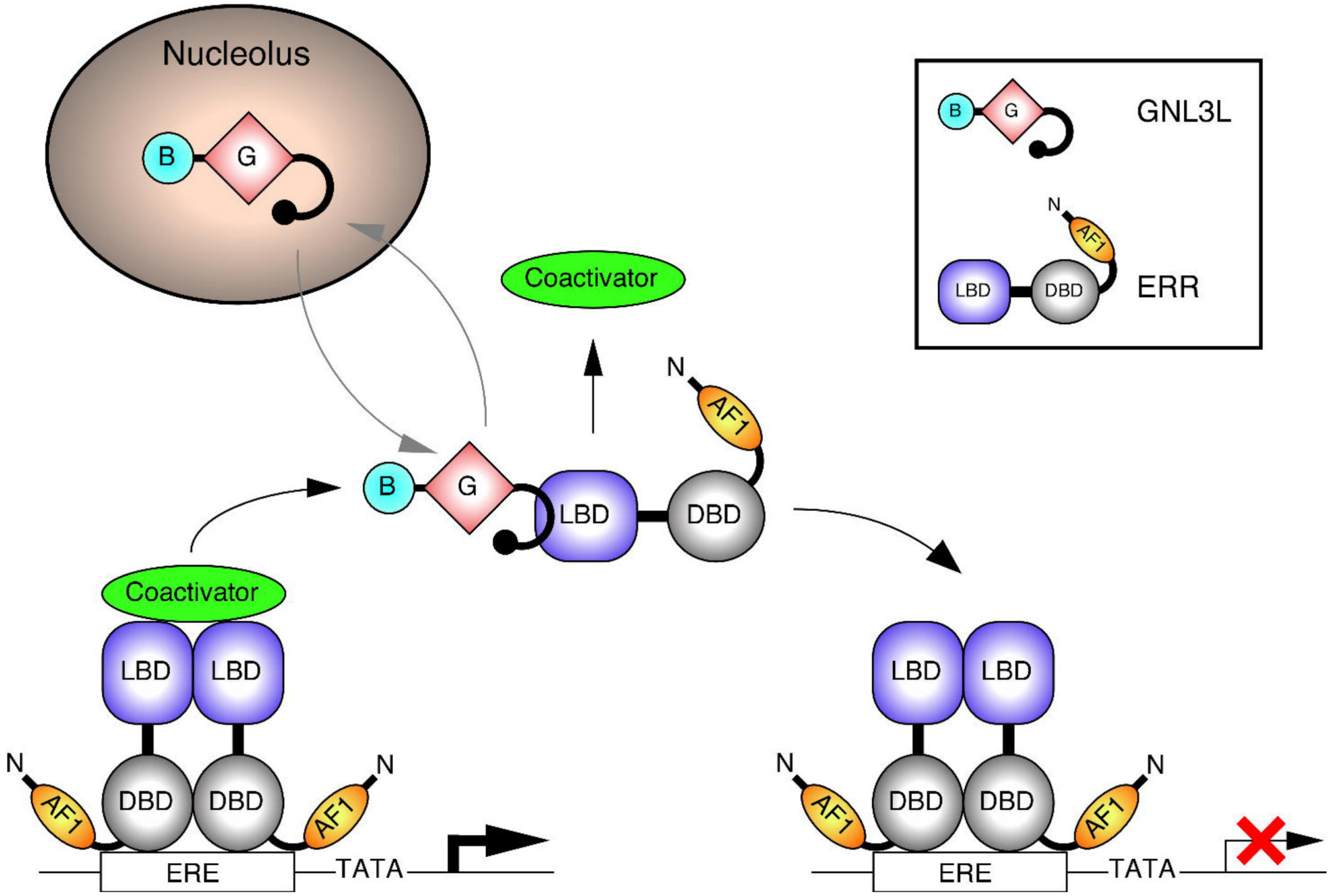

Figure 9. GNL3L inhibits the transcriptional activities of ERR family genes by coactivator competition

Our data reveal a novel mechanism that regulates the activity of ERR family genes by a nucleolar GTP-binding protein GNL3L. GNL3L decreases the transcriptional activity of ERR proteins. This event takes place in the nucleoplasm and does not require the nucleolar localization of GNL3L. The interaction between GNL3L and ERRγ displaces coactivators such as SRC1 and SRC2 from the ERRγ complex. The SRC-depleted ERRγ protein binds DNA without GNL3L, resulting in transcriptional inhibition. In this model, the nucleolar accumulation of GNL3L does not appear to affect its ability to suppress the transcriptional function of ERR proteins (grey arrows). Abbreviations for protein domains of GNL3L and ERR are explained in Fig. 2A and 2D.