
Figures and figure supplements

tRNA synthetase counteracts c-Myc to develop functional vasculature

Yi Shi, et al.

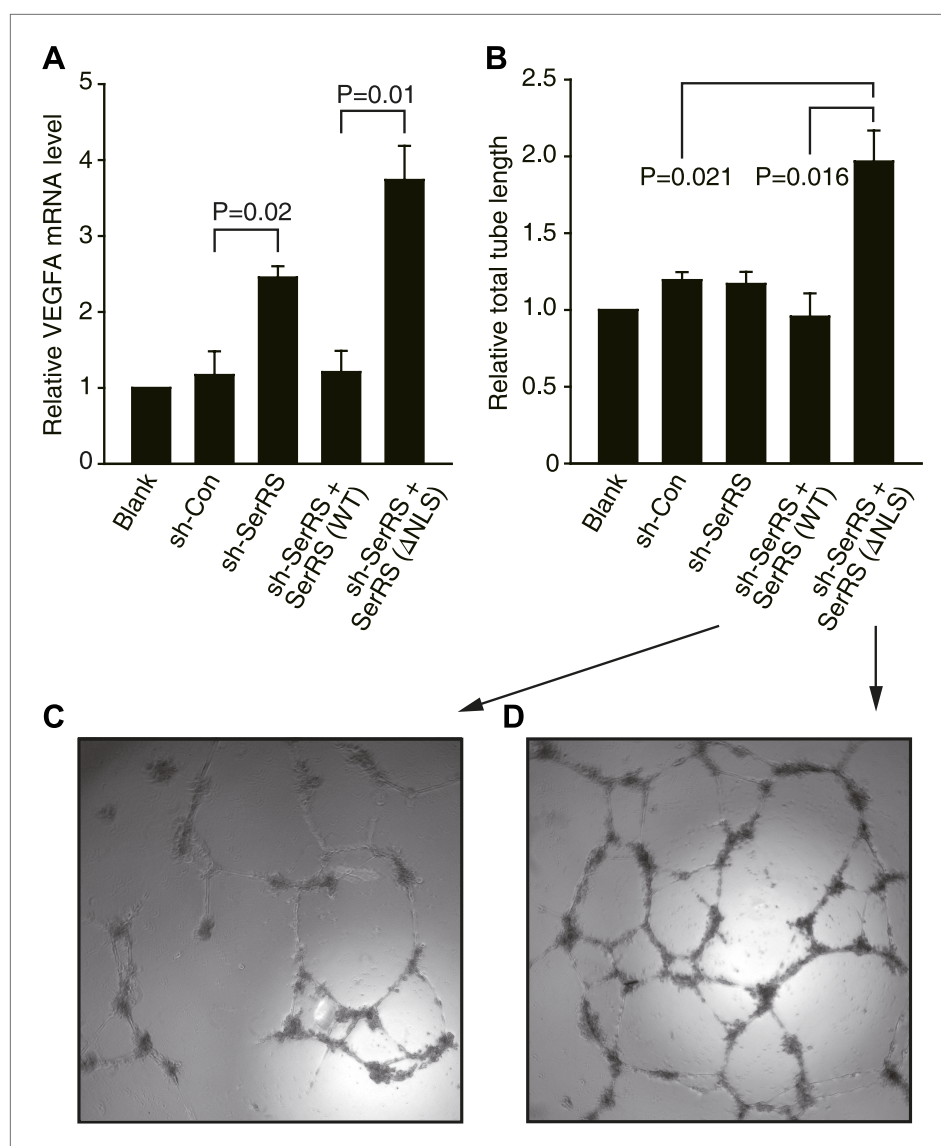


Figure 1. Nuclear SerRS suppresses VEGFA expression and angiogenesis. **(A)** VEGFA mRNA levels as detected by real-time RT-qPCR in HUVECs infected with lentiviral plasmids expressing nonspecific control shRNA (sh-Con), SerRS-specific shRNA (sh-SerRS), or sh-SerRS and wild type (WT) or NLS-deleted (Δ NLS) SerRS simultaneously. Values are means $\pm$ SEM ($n = 3$). **(B)** Endothelial tube formation assay to show that excluding SerRS from the nucleus promotes angiogenesis. Values are means $\pm$ SEM ($n = 3$). **(C and D)** Representative images of the tubular network formed by HUVECs expressing WT and Δ NLS SerRS, respectively.

DOI: [10.7554/eLife.02349.003](https://doi.org/10.7554/eLife.02349.003)

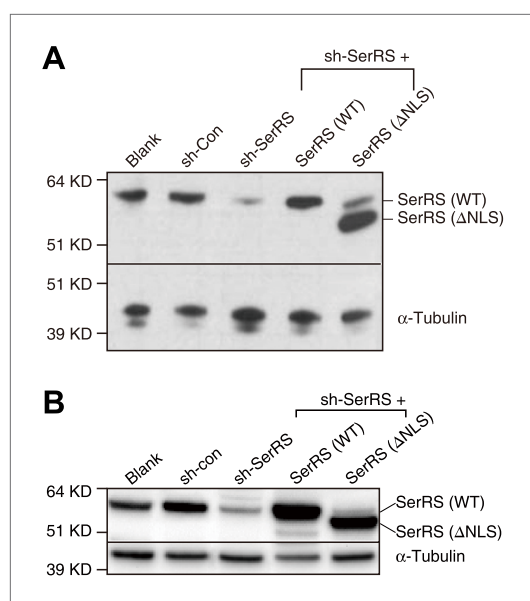


Figure 1—figure supplement 1. Manipulations of the expression of SerRS in HUVEC and HEK 293 cells.

DOI: [10.7554/eLife.02349.004](https://doi.org/10.7554/eLife.02349.004)

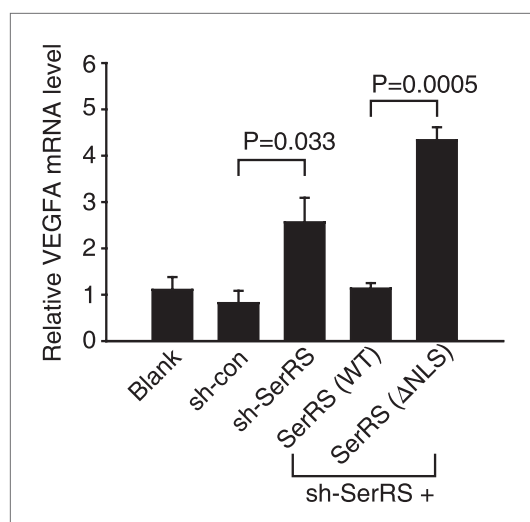
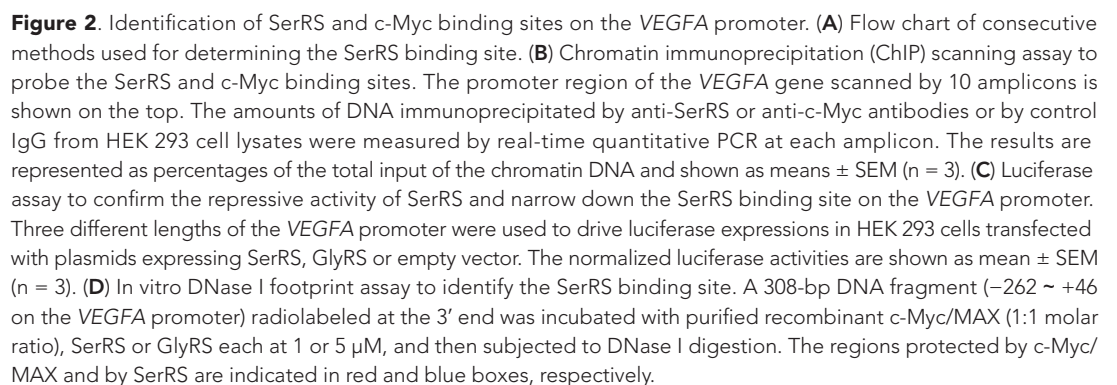


Figure 1—figure supplement 2. Nuclear SerRS suppresses VEGFA expression in HEK 293 cells.

DOI: [10.7554/eLife.02349.005](https://doi.org/10.7554/eLife.02349.005)



4 of 16

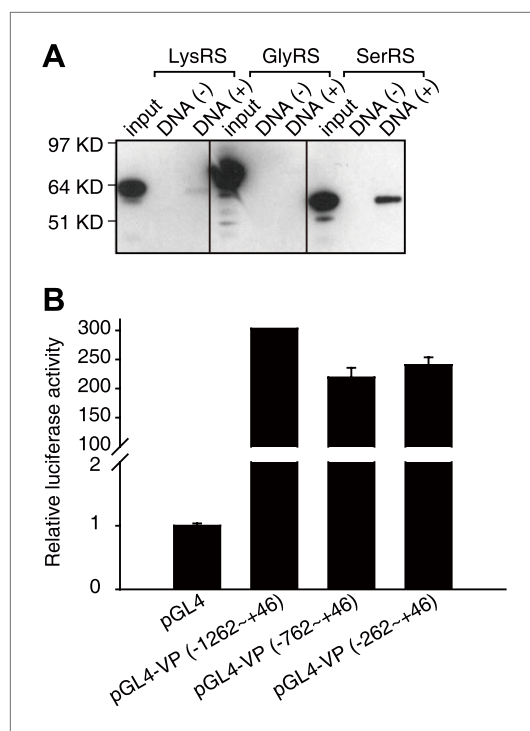


Figure 2—figure supplement 1. Identification of the interaction between SerRS and DNA.

DOI: [10.7554/eLife.02349.007](https://doi.org/10.7554/eLife.02349.007)

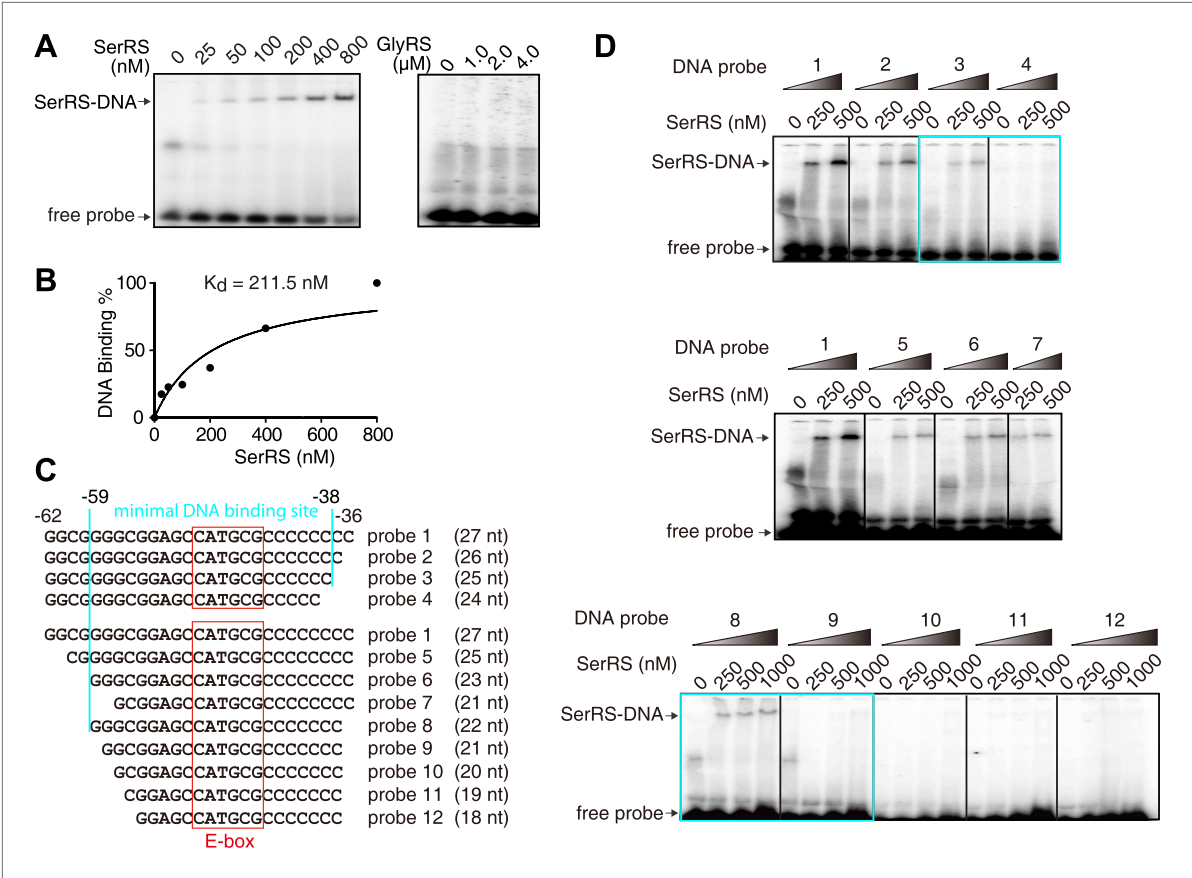


Figure 3. Characterization of the interaction between SerRS and DNA. **(A and B)** In vitro EMSA assay to determine the binding affinity between SerRS and the 27-bp DNA. The 27-bp DNA fragment containing SerRS binding site on the *VEGFA* promoter (−62 ~ −36) were labeled by ³²P at the 5' end, and then incubated with purified SerRS or GlyRS at indicated concentrations. The SerRS–DNA complex was followed by electrophoresis on native acrylamide gels. **(C and D)** EMSA to determine the minimal SerRS binding site on the *VEGFA* promoter. Truncations of the DNA from either end weakened the SerRS–DNA interaction. Purified recombinant SerRS protein was used at the indicated concentrations.

DOI: 10.7554/eLife.02349.008

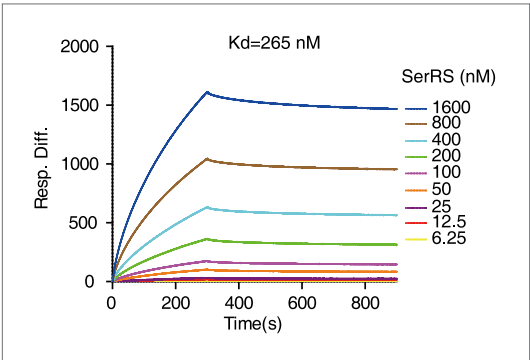


Figure 3—figure supplement 1. Determination of the binding affinity between SerRS and DNA by SPR.

DOI: 10.7554/eLife.02349.009

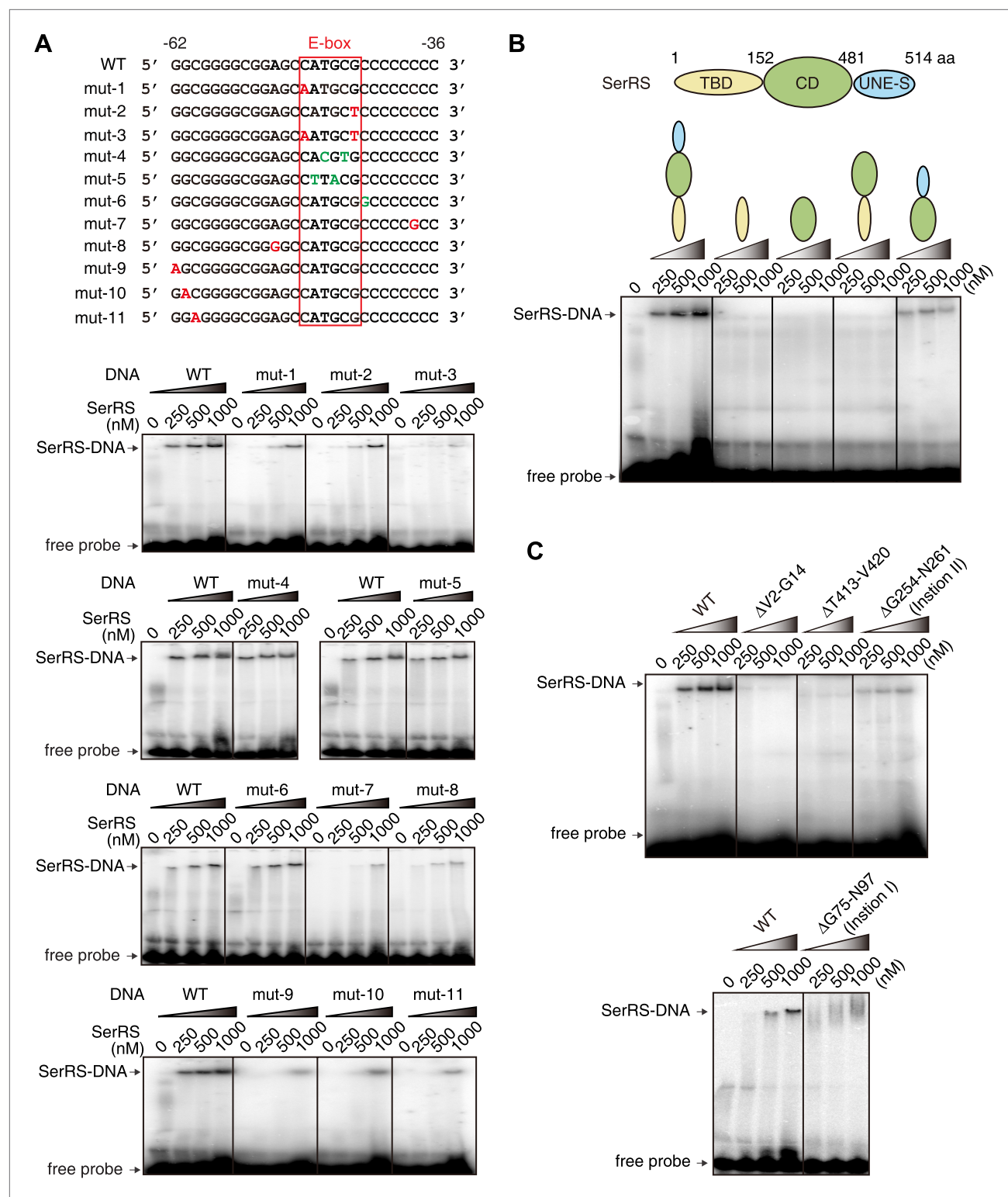


Figure 4. Further characterization of the interaction between SerRS and DNA. **(A)** EMSA assay to probe the DNA sequence specificity for interacting with SerRS. DNA mutations that do or do not impact SerRS binding are colored in red and green, respectively. **(B)** Domain mapping analysis and EMSA assay to reveal multiple DNA binding sites on SerRS. TBD: tRNA binding domain; CD: catalytic domain; UNE-S: C-terminal appended domain unique to vertebrates. **(C)** Deletion mutagenesis to further define DNA binding sites on SerRS. Deletion of either insertion I, insertion II, motif V2-G14, or loop T413-V420 greatly weakens or abolishes the DNA interaction.

DOI: [10.7554/eLife.02349.010](https://doi.org/10.7554/eLife.02349.010)

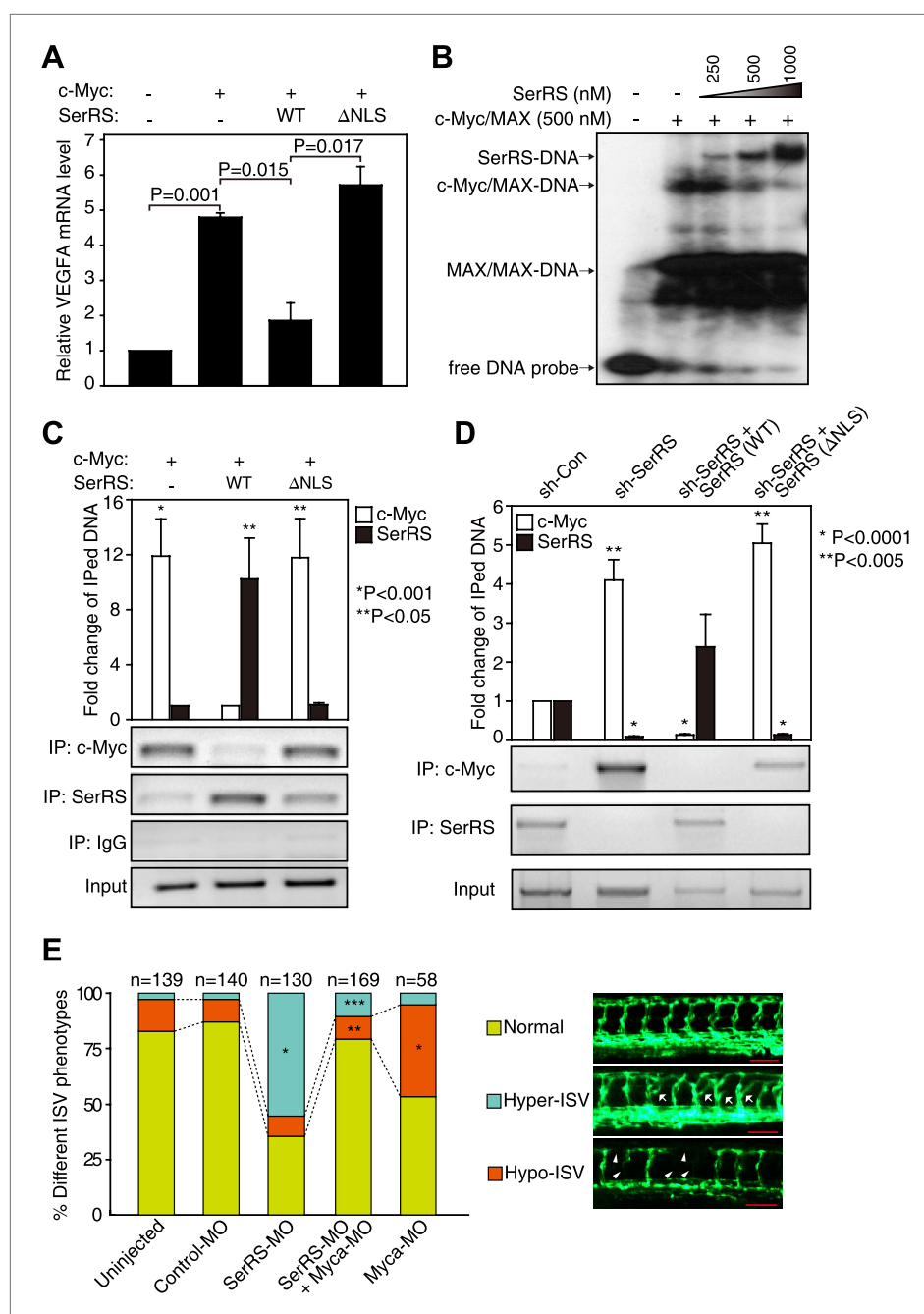


Figure 5. Competition between SerRS and c-Myc for DNA binding and their opposing effect in vascular development. **(A)** Competition between c-Myc and SerRS on VEGFA expression. HEK 293 cells were transfected with c-Myc alone or c-Myc with WT or ΔNLS SerRS. The mRNA levels of VEGFA were determined by RT-PCR. Values are shown as means ± SEM (n = 3). **(B)** Competition between c-Myc/MAX and SerRS for DNA binding in vitro as examined by EMSA. The 27-bp DNA was radio-labeled and incubated with purified recombinant c-Myc/MAX together with purified recombinant SerRS at indicated concentrations. The protein–DNA complexes were followed by electrophoresis on a native acrylamide gel. **(C)** Competition between ectopically expressed SerRS and c-Myc for DNA binding on the VEGFA promoter in HEK 293 cells as examined by ChIP. HEK 293 cells were co-transfected with plasmids expressing c-Myc and WT or ΔNLS SerRS or empty vector (–) 24 hr prior to ChIP analysis. The amounts of DNA immunoprecipitated by anti-SerRS or anti-c-Myc antibodies or by control IgG from HEK 293 cell lysates were measured by PCR using a primer set targeting the VEGFA promoter. The normalized results (top panel) are represented as fold change of immunoprecipitated DNA by anti-SerRS vs anti-c-Myc and are shown as means ± SEM (n = 3, *p<0.001, **p<0.05). *Figure 5. Continued on next page*

Figure 5. Continued

The bottom panel shows representative gel images. **(D)** Competition between endogenously expressed SerRS and c-Myc for DNA binding on the *VEGFA* promoter in HUVECs. HUVECs were infected to express the indicated molecules 48 hr prior to ChIP analysis. The same ChIP experiment and data analysis were performed as described in **(C)**. * $p < 0.0001$, ** $p < 0.005$. **(E)** Opposing effect of SerRS and c-Myc in zebrafish vascular development and their mutual phenotypic rescue. The percentage of *Tg(Fli1a:GFP)* zebrafish embryos showing different ISV phenotypes at 3 days post fertilization after the injection of morpholinos targeting SerRS (SerRS-MO), Myca (Myca-MO), or a control morpholino (Control-MO) are illustrated. Scale bars represent 0.125 mm. * $p < 0.0001$ vs Control-MO, ** $p < 0.0001$ vs Myca-MO, *** $p < 0.0001$ vs SerRS-MO. Control-MO was added to SerRS-MO or Myca-MO experiments in order to maintain a constant level of total morpholinos in each experiment.

DOI: [10.7554/eLife.02349.012](https://doi.org/10.7554/eLife.02349.012)

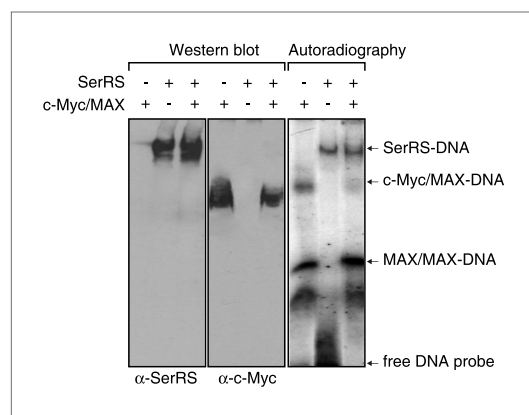


Figure 5—figure supplement 1. SerRS and c-Myc/MAX do not simultaneously bind to the DNA.

DOI: [10.7554/eLife.02349.013](https://doi.org/10.7554/eLife.02349.013)

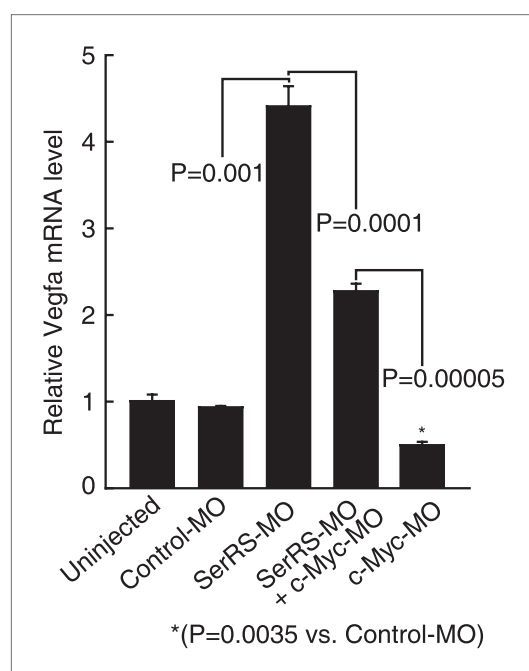


Figure 5—figure supplement 2. Effect of knocking down Myca or SerRS on Vegfa expression in zebrafish.

DOI: [10.7554/eLife.02349.014](https://doi.org/10.7554/eLife.02349.014)

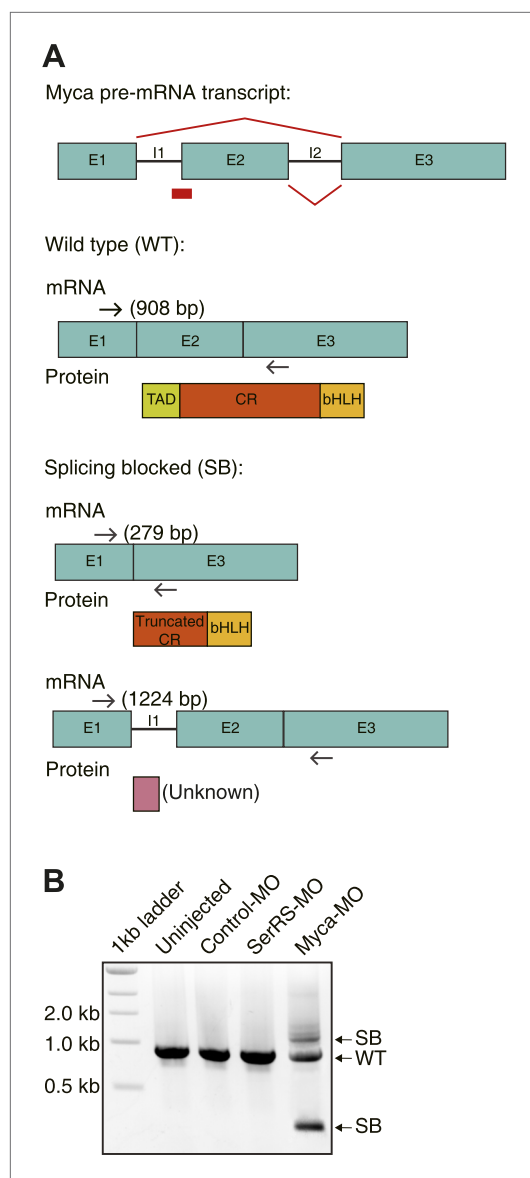


Figure 5—figure supplement 3. Design and efficiency of the antisense morpholino against Myca.

DOI: [10.7554/eLife.02349.015](https://doi.org/10.7554/eLife.02349.015)

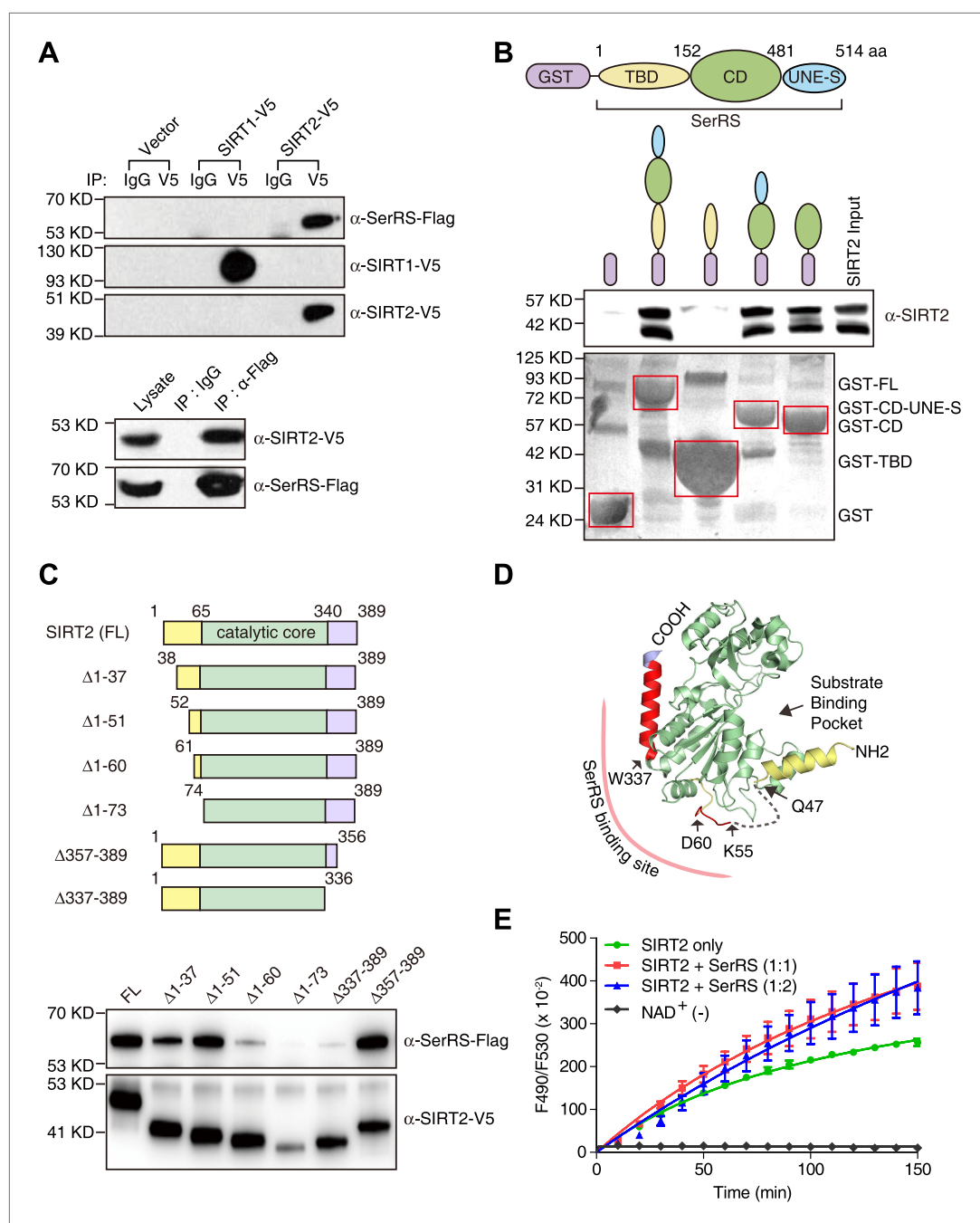


Figure 6. Demonstration and characterization of SerRS/SIRT2 interaction. **(A)** SerRS specifically interacts with SIRT2 but not SIRT1. HEK 293 cells were co-transfected with plasmids expressing Flag-tagged SerRS and V5-tagged SIRT1 or SIRT2. Cell lysate was immunoprecipitated with anti-V5 (top panel), anti-Flag (bottom panel) antibodies or control IgG. The experiment was followed by Western blot analysis to detect the interaction between SerRS and SIRT1/SIRT2 using anti-Flag and anti-V5 antibodies. **(B)** GST-pull down assay to show that SerRS/SIRT2 interaction is direct and that the interaction is mediated by the catalytic domain of SerRS. Full-length SerRS or its domain fragments were fused with GST at N-termini to pull down purified His-tagged SIRT2. SIRT2 was detected by Western blot analysis using anti-His₆ antibody, and the GST fusion proteins attached on the Glutathione-Sepharose beads were analyzed using ponceau S staining. TBD: tRNA-binding domain; CD: catalytic domain; UNE-S: C-terminal appended domain. **(C)** Mapping study to identify the SerRS binding sites on SIRT2. V5-tagged full-length SIRT2 or its truncated fragments was co-transfected with Flag-tagged SerRS into HEK 293 cells. SIRT2 proteins were immunoprecipitated with anti-V5 antibody and the SIRT2-bound SerRS proteins were detected by Western blot using anti-Flag antibody. *Figure 6. Continued on next page*

Figure 6. Continued

(D) Illustration of the SerRS binding sites on the structure of SIRT2. Two SerRS binding sites (Gly52-Asp60, Trp337-Ser356) are highlighted in red. The catalytic domain of SIRT2 is in green, while the partially disordered N- and C-terminal regions are in yellow and purple, respectively. The gray dash line represents a disordered internal region. (E) Effect of SerRS on SIRT2 deacetylation activity. Recombinant human SIRT2 (1 μ M) were incubated with purified SerRS (concentration measured as monomer) at the indicated ratios. The deacetylase activities of SIRT2 were measured by using a substrate peptide with one end coupled to a fluorophore and the other end to a quencher. An internal acetylated lysine residue serves as the substrate of SIRT2, and the deacetylation allows the peptide to be cleaved by a lysylendopeptidase to release the fluorophore from the quencher to emit fluorescence. Therefore, the SIRT2 activity was measured by monitoring the fluorescence intensity (excitation at 490 nm and emission at 530 nm). A reaction without NAD^+ (NAD^+ [–]) was performed as a negative control.

DOI: [10.7554/eLife.02349.016](https://doi.org/10.7554/eLife.02349.016)

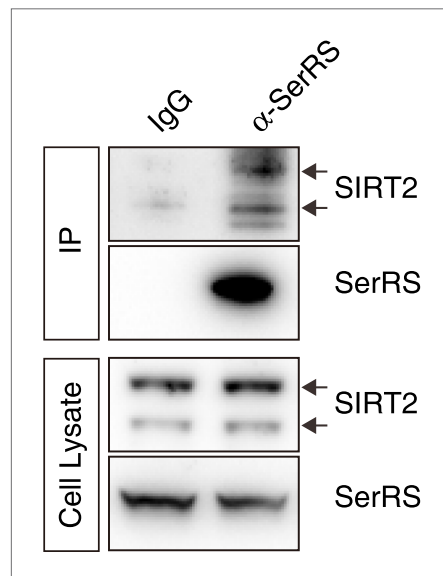


Figure 6—figure supplement 1. Endogenous interaction between SerRS and SIRT2.

DOI: [10.7554/eLife.02349.017](https://doi.org/10.7554/eLife.02349.017)

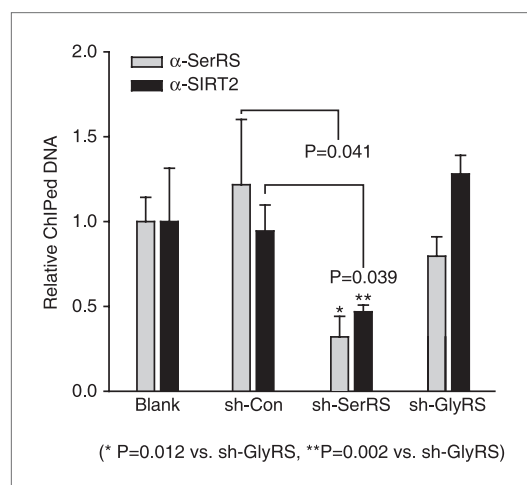


Figure 6—figure supplement 2. SerRS recruits SIRT2 to the VEGFA promoter.

DOI: [10.7554/eLife.02349.018](https://doi.org/10.7554/eLife.02349.018)

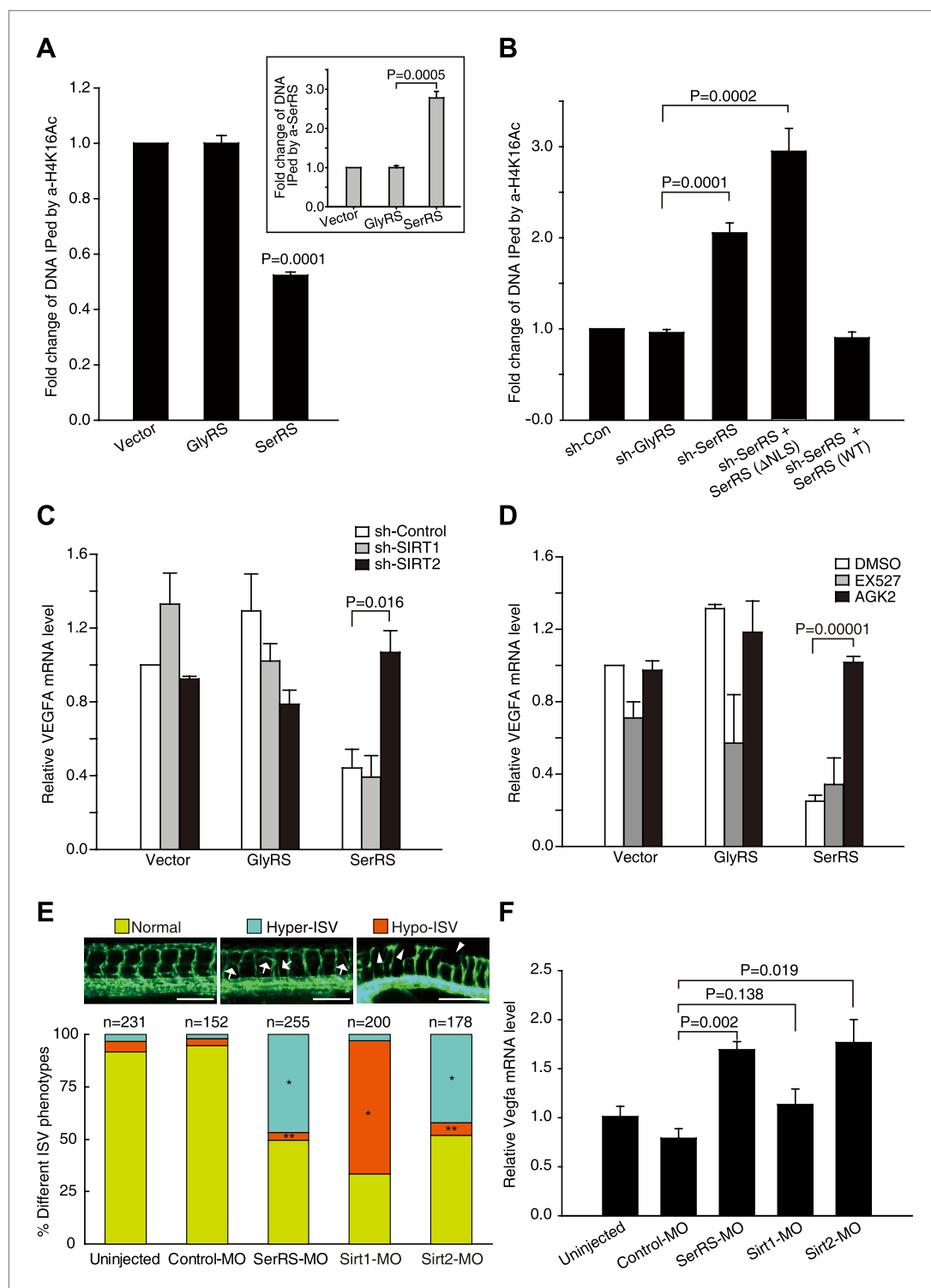


Figure 7. SerRS recruits SIRT2 to epigenetically silence VEGFA expression. **(A)** ChIP assay to show that overexpression of SerRS reduces histone H4 acetylation level on the VEGFA promoter. HEK 293 cells were transfected with plasmids expressing SerRS, GlyRS or empty vector. The cell lysates were subjected to local ChIP analysis using anti-H4K16Ac (acetylated H4 at K16), anti-H4 (total), or anti-SerRS antibodies and a primer set targeting the VEGFA promoter. The amounts of DNA immunoprecipitated by anti-H4K16Ac antibody were normalized to those by anti-H4 antibody prior to fold change calculation. Inset: the normalized amounts of DNA immunoprecipitated by anti-SerRS. All data were shown as means $\pm$ SEM ($n = 3$). **(B)** ChIP assay to show that knock down of SerRS expression or exclusion of

Figure 7. Continued on next page

Figure 7. Continued

SerRS from the nucleus increases histone H4 acetylation level on the *VEGFA* promoter. HEK 293 cells were transfected with plasmids expressing the indicated molecules and subjected to local ChIP analysis as described above. As a control, GlyRS expression was knocked down but had no effect on H4 acetylation. **(C)** Effect of SIRT2 expression on the transcriptional repressor activity of SerRS as measured by *VEGFA* expression. HEK 293 cells were co-transfected with plasmids expressing shRNAs targeting SIRT1, SIRT2 or control shRNA and plasmids expressing SerRS, GlyRS or empty vector for 36 hr. The *VEGFA* expression levels were determined by using real-time RT-qPCR and are shown as means $\pm$ SEM ($n = 3$). **(D)** Effect of SIRT2-specific inhibitor on the transcriptional repressor activity of SerRS as measured by *VEGFA* expression. HEK 293 cells were transfected with plasmids expressing SerRS, GlyRS or empty vector. SIRT2-specific inhibitor AGK2 (10 μ M, final concentration) or SIRT1-specific inhibitor EX-527 (1 μ M, final concentration) or solvent alone (DMSO) was added to the cell culture media 2 hr post-transfection. *VEGFA* expression levels were measured 24 hr post-transfection by using real-time RT-qPCR and are shown as means $\pm$ SEM ($n = 3$). **(E)** Functional correlation between SerRS and SIRT2 in zebrafish. The percentage of *Tg(Fli1a:EGFP)* zebrafish embryos showing different ISV phenotypes at 3 days post fertilization after the injection of morpholinos targeting SerRS (SerRS-MO), Sirt1 (Sirt1-MO), Sirt2 (Sirt2-MO), or a control morpholino (Control-MO) are illustrated. Scale bars represent 0.25 mm. * $p < 0.0001$ vs Control-MO, ** $p > 0.1$ vs Control-MO. **(F)** The effects of knocking down SerRS, Sirt2, or Sirt1 in zebrafish on *Vegfa* expression were examined by real-time RT-qPCR at 1 day post fertilization after injection of morpholinos as indicated. Data are shown as means $\pm$ SEM ($n = 10-15$).

DOI: [10.7554/eLife.02349.019](https://doi.org/10.7554/eLife.02349.019)

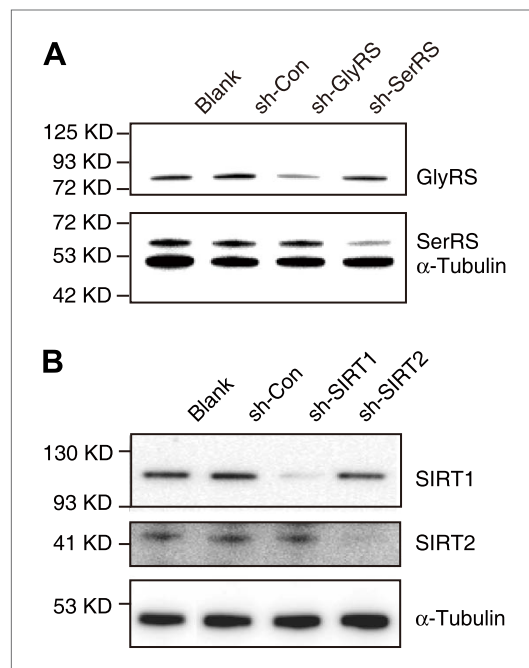


Figure 7—figure supplement 1. Knock-down efficiencies of shRNAs targeting GlyRS, SerRS, SIRT1, and SIRT2.

DOI: [10.7554/eLife.02349.020](https://doi.org/10.7554/eLife.02349.020)

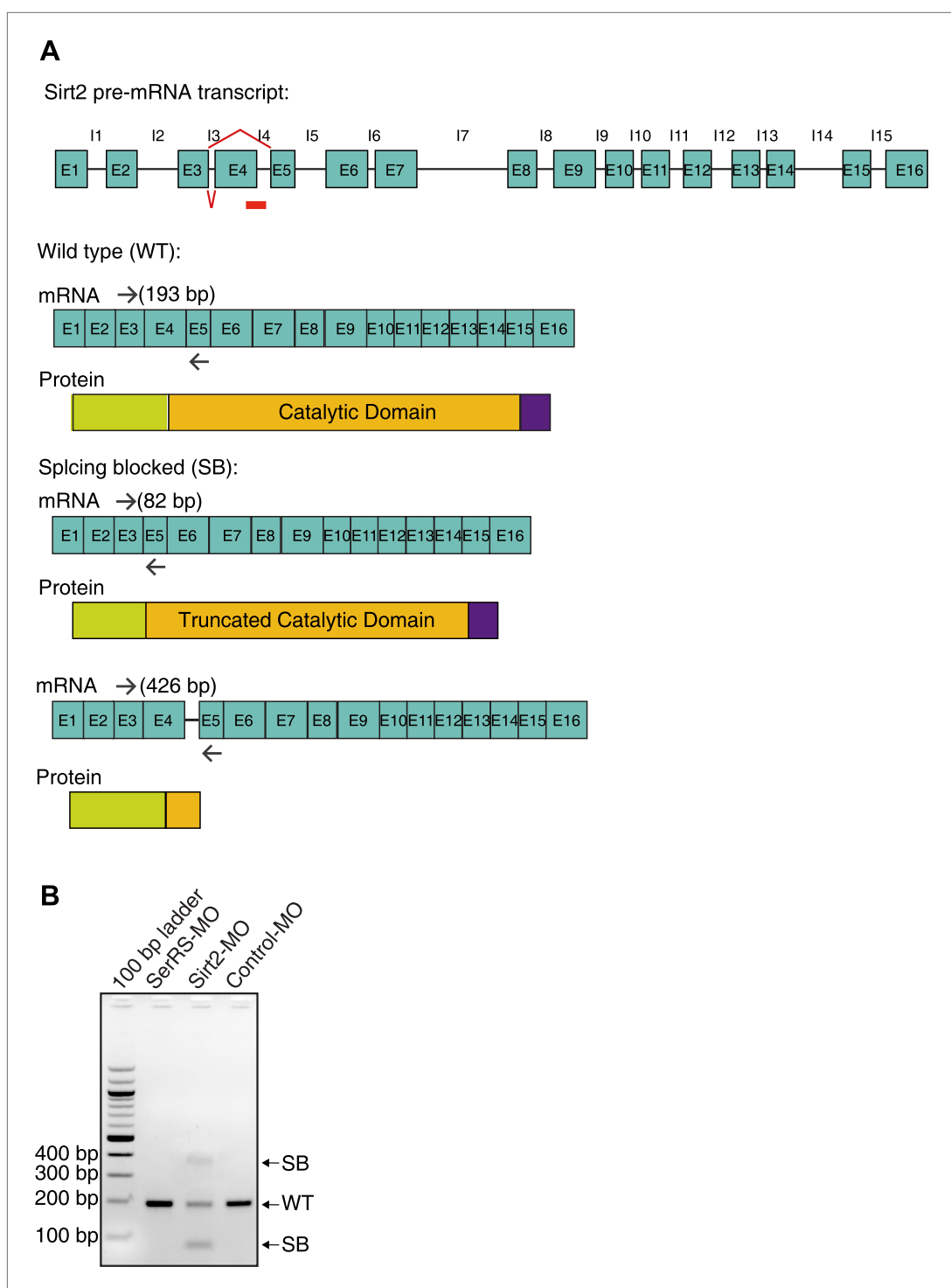


Figure 7—figure supplement 2. Design and efficiency of the antisense morpholino against Sirt2.

DOI: [10.7554/eLife.02349.021](https://doi.org/10.7554/eLife.02349.021)

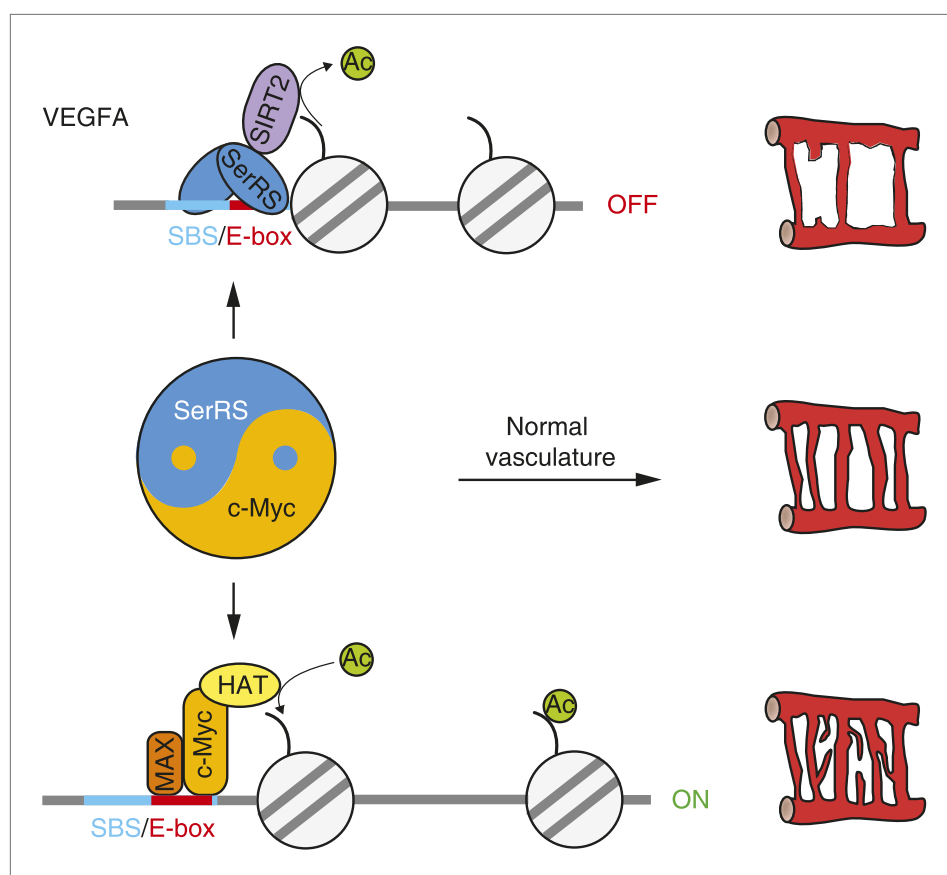


Figure 8. The ‘Yin-Yang’ relationship of SerRS and c-Myc in vascular development. Nuclear SerRS binds to the VEGFA promoter at the identified SerRS binding site (SBS) and recruits the SIRT2 histone deacetylase to condense the local chromatin to shut down VEGFA expression. These tandem actions of SerRS symmetrically offset the VEGFA-promoting actions of c-Myc to maintain a delicate balance for the development of a functional vasculature. DOI: [10.7554/eLife.02349.022](https://doi.org/10.7554/eLife.02349.022)