New domains were progressively added to cytoplasmic aminoacyl transfer RNA (tRNA) synthetases during evolution. One example is the UNE-S domain, appended to seryl-tRNA synthetase (SerRS) in species that developed closed circulatory systems. Here we show using solution and crystal structure analyses and in vitro and in vivo functional studies that UNE-S harbours a robust nuclear localization signal (NLS) directing SerRS to the nucleus where it attenuates vascular endothelial growth factor A expression. We also show that SerRS mutants previously linked to vasculature abnormalities either deleted the NLS or have the NLS sequestered in an alternative conformation. A structure-based second-site mutation, designed to release the sequestered NLS, restored normal vasculature. Thus, the essential function of SerRS in vascular development depends on UNE-S. These results are the first to show an essential role for a tRNA synthetase-associated appended domain at the organism level, and suggest that acquisition of UNE-S has a role in the establishment of the closed circulatory systems of vertebrates.
Aminoacyl transfer RNA (tRNA) synthetases (aaRSs) are enzymes essential for translation throughout the three kingdoms of life\(^1\). Each member of the tRNA synthetase family is responsible for charging one specific amino acid onto its cognate tRNA. Once the tRNA is charged and delivered to the ribosome, the amino acid can be transferred from the tRNA onto a growing peptide according to the genetic code. During the evolution of this ancient protein family, new domains and motifs were progressively added to aaRSs to expand their functionalities\(^3\). These appended domains, often dispensable for aminoacylation, are considered as markers for the aaRS-associated functions beyond translation\(^5\). Although their appearance correlates with the increase of biological complexity in higher organisms, the functional significance of these aaRS-associated appended domains is not understood at the organism level.

With this question in mind, we recently identified a unique domain (named UNE-S) at the carboxyl-terminus of seryl-tRNA synthetase (SerRS) in all vertebrates from fish to humans\(^6\). Interestingly, three independent forward-genetic studies in zebrafish suggested a role for SerRS in vascular development\(^7\). Disruption of the gene encoding the cytoplasmic SerRS by insertional mutagenesis results in a null mutation, and causes abnormal blood vessel formation and defective blood circulation in the embryo\(^8\). Separately, two additional studies identified three N-ethyl-N-nitrosourea-induced mutations in SerRS that also cause vasculature defects\(^9\). Two of these mutations result in premature stop codons (Q402Stop and E421Stop) eliminating part of the aminoacylation domain and the entire UNE-S domain, while the third is a missense mutation (F383V) located in the aminoacylation domain and allows expression of the full enzyme. Microinjection of either human or zebrafish SerRS messenger RNA (mRNA) into ko095 (Q402Stop) mutant zebrafish embryos rescued the vascular phenotype, indicating that the vascular role of SerRS is conserved between zebrafish and humans\(^8\). Interestingly, an aminoacylation-defective SerRS (T429A SerRS) mRNA could also restore the vasculature phenotype, suggesting that the role of SerRS in vasculature development is independent of aminoacylation\(^5\). This noncanonical activity of SerRS was linked to the expression of vegfa, the gene encoding vascular endothelial growth factor A (VEGFA), a key regulator of angiogenesis and vasculogenesis\(^8\).

Considering that UNE-S was joined to SerRS at the time of development of the closed circulatory system, we speculated that UNE-S is relevant to the noncanonical role of SerRS in vascular development. In this study, we identified a nuclear localization signal (NLS) sequence embedded in UNE-S, and designed a series of experiments to demonstrate that the essential role of SerRS in vascular development is dependent on UNE-S and its role to mobilize SerRS from the cytoplasm to the nucleus.

**Results**

UNE-S-dependent nuclear localization of SerRS. From inspection of UNE-S, we identified a **KKK** sequence as a putative NLS sequence that is highly conserved in all vertebrates (Fig. 1). This observation raised the possibility that, in addition to being present in the cytoplasm for protein synthesis, SerRS might...
also be distributed to the nucleus. Confocal immunofluorescence microscopy demonstrated nuclear localization of SerRS in human endothelial cells (that is, HUVECs) (Fig. 2a). Cell fractionation analysis further confirmed the nuclear distribution of SerRS in HUVECs (Fig. 2b). Nuclear distribution of endogenous SerRS was also detected in HEK 293 T cells (Fig. 2b), indicating that the mechanism for bringing SerRS into the nucleus is not restricted to endothelial cells. We estimated that for both cell types, ~10% of cellular SerRS is located in the nucleus based on our western blot analysis (Fig. 2b).

The potential NLS in UNE-S has the profile of a bipartite localization signal with two stretches of positively charged lysines that could be recognized by a nuclear transport protein10,13. To test its authenticity as an NLS, we added the corresponding sequence from human SerRS (482-KKQKKHEGSKKK-494) to the carboxy-terminus of the green fluorescent protein (GFP). The GFP–NLS fusion protein, when expressed in HeLa or HEK 293 T cells, predominantly localized to the nucleus (Fig. 2c). In contrast, the native GFP protein localized both to the nucleus and to the cytosol. These results show that the predicted NLS sequence in UNE-S is sufficient to direct nuclear localization.

To establish whether the 482-KKQKKHEGSKKK-494 NLS sequence is necessary for nuclear localization of SerRS, we deleted or mutated the NLS and compared the nuclear localization of the mutants with that of wild-type (WT) SerRS. As expected, the transfected WT protein was distributed in the nucleus of HEK 293 T cells (Fig. 3a). We then created two deletion mutations that removed either the entire UNE-S (Δ470–514) or only the region from the NLS to the C-terminus (Δ482–514). In contrast to the WT SerRS, both deletion constructs only appeared in the cytoplasm and had no detectable nuclear distribution (Fig. 3a). We also created mutants that replace lysine residues in the NLS with alanines. As the number of lysine substitutions increased, the amount of SerRS nuclear localization decreased. A single lysine mutant K493A had a slightly reduced nuclear localization (data not shown), but only trace amounts of the triple mutant K482A/K485A/K493A (designated as NLSmut) were detected in the nucleus (Fig. 3a). These results strongly suggest that the predicted bipartite NLS in UNE-S is critical for the nuclear localization of SerRS.

SerRS mutants are defective in nuclear localization. Among the three different mutations in the SerRS gene (SARS) that are linked to zebrafish vasculature abnormalities, two were nonsense mutations resulting in a premature stop codon after Y401 or V4208,9 yielding C-terminal truncated SerRS proteins lacking UNE-S (Fig. 1). Based on our data above, neither of these truncated proteins would be localized to the nucleus. The question remains on the third mutation, which results in a F383V substitution that is located ~100 amino acids upstream of the NLS. Interestingly, this F383V substitution completely abolishes nuclear localization of SerRS (Fig. 3b). Thus, all three SerRS mutations linked to vasculature abnormalities in zebrafish would result in defective nuclear localization of SerRS. In contrast, the aminoacylation-defective T429A SerRS, which rescued the abnormal vascular phenotype, localizes to the nucleus as efficient as WT SerRS (Fig. 3c). The correlation between vasculature abnormality and the lack of SerRS nuclear localization strongly suggests that the role of SerRS in vascular development is dependent on the NLS embedded in UNE-S.

Crystal structure analysis of human SerRS. To understand how F383V affects nuclear localization, we determined the crystal structure of human SerRS at 2.9 Å resolution (Fig. 4a and Supplementary Table S1). The human protein shares overall 81% sequence identity with the fish ortholog, and F383 is a strictly conserved residue from fish to humans (Fig. 1 and Supplementary

Figure 3 | UNE-S-dependent SerRS nuclear localization and its disruption by a mutation linked to vasculature abnormality. (a) Cell fractionation and mutagenesis studies showing that the predicted NLS within the UNE-S domain is responsible for the nuclear localization of SerRS. Subcellular localizations of the exogenously expressed SerRS proteins were detected by anti-flag tag polyclonal antibody. Deletion of the whole UNE-S domain (Δ470–514) or deletion from the NLS (Δ482–514) abolished the nuclear localization of SerRS. Triple point mutation within the NLS (K482A/K485A/K493A, designated as NLSmut) significantly reduced the nuclear localization. (b) Cell fractionation analysis demonstrating that the F383V mutant linked to vasculature abnormality in zebrafish is defective in nuclear localization. (c) Cell fractionation analysis confirms the intact nuclear localization of T429A SerRS. Fig. S1). Three independent homodimers of SerRS were found in the asymmetric unit of the crystal. While the conformation of the aminoacylation domain is almost the same for all three dimers in the asymmetric unit, the N-terminal tRNA-binding domains have more flexible structures (Fig. 4b). Interestingly, the C-terminal UNE-S domain (including the NLS) was mostly disordered in all six subunits, suggesting a dynamic conformation of the NLS that would enhance its accessibility to the nuclear transport machinery. F383 is located near the end of a β-strand (β10) that is part of the core seven-stranded antiparallel β-sheet (β1–β9–β10–β11–β13–β8–β7) of the aminoacylation domain, and spatially close to the active site and the flexible NLS (Fig. 4a,c). The side chain of F383 forms hydrophobic interactions with H170 and F316 to stabilize the β10–β11 hairpin as part of the central core (Fig. 4c). A stereo image of the electron density map surrounding F383 is shown in Figure 4d. We speculated that the F383V substitution would destabilize the hydrophobic core and, in some way, create an internal binding site for the NLS. As a result, the NLS would become less accessible, as illustrated in Figure 4c, and less able to facilitate nuclear localization.
The peptide covering the F383V mutation site (E380-L392) is the only area on the protein that exhibits a dramatic increase in HDX (41% over WT SerRS) (Supplementary Fig. S2 and Fig. S3a), presumably because of the weakened hydrophobic core caused by the mutation. Remarkably, also as a result of the F383V mutation, the NLS region shows the most dramatic decrease in HDX (64% below WT SerRS), indicating that it is stabilized in the mutant. This result is consistent with our hypothesis that the NLS becomes less accessible in the F383V mutant (Figs 4c and 5a).

**Structure-based design of second-site revertant of F383V.** We next wanted to determine the F383V-induced internal binding site for the NLS. By analysing the surface electrostatic potential of SerRS, three negatively charged sites were identified as potential binding sites for the positively charged NLS. Those sites are spatially adjacent to both the F383V mutation site and the partially resolved UNE-S in the crystal structure. As shown in Supplementary Figure S3a, site I contains four negatively charged residues (D148, E149, E150 and D152), site II contains D178, E181 and E183, while site III contains D378, E380 and E391. Among them, site III is the closest to the F383V mutation site (Supplementary Fig. S3b). If some of those negatively charged residues are important for NLS binding,
their substitution with neutral or positively charged residues would release the NLS and restore nuclear localization.

To identify the NLS-binding site on F383V SerRS, we created mutations within each site (I or II or III) on top of the F383V background. While mutations within sites I and II did not have any effect on nuclear localization (Supplementary Fig. S3c), a double mutation within site III (D378S/E380G) partially rescued the nuclear localization defect of F383V (Supplementary Fig. S3c). The rescue effect was mostly contributed by the D378S mutation alone, while substitution of D378 with a positively charged arginine provided further rescue (Fig. 5b). These results suggest that D378 has a major role in tethering the NLS in F383V SerRS, and that this tethering effect is largely dependent on electrostatic interactions. Consistently, as a result of the NLS binding, the D378-containing peptide (V365-D378) exhibited a 23% decrease in HDX in F383V SerRS relative to WT SerRS. Deuterium uptake time-course curves for representative peptides are shown on the right. (b) Cell fractionation experiment showing that substitution of D378 with S or R rescues the nuclear localization deficiency of F383V SerRS. (c) Aminoacylation and ATP-PPi exchange assays showing that the NLS is dispensable for aminoacylation and the F383V mutant remains partially active. The partial activity of F383V SerRS is completely lost when the NLS is deleted or released by the second mutation D378R. Error bars represent the s.e.m of triplicate experiments.

Role of UNE-S in vascular development in the zebrafish. To confirm a functional link between the NLS and vascular development in a vertebrate model, we tested the rescue of vascular abnormality in zebrafish by various SerRS mutants. A fish model for vasculature abnormalities was created by injecting an antisense morpholino (MO) directed against SerRS(5). Consistent with the previous report by Fukui et al.(5), we show that injection of SerRS-MO results in abnormal intersegmental vessel (ISV) branching in 50.8% (% = 96 out of 189) of morphants, in contrast to 1.4% (% = 3 out of 207) in un.injected controls (Fig. 6a and Table 1). Co-injection of human SerRS mRNA efficiently rescued the abnormal ISV branching (8.4%; % = 16 out of 190) in SerRS morphants (Fig. 6a and Table 1). In contrast, NLSmut and F383V SerRS, which we showed to be defective in nuclear localization (Fig. 3a,b), could not rescue the aberrant ISV branching (Fig. 6b,c and Table 1). Abnormal ISV branching was observed in 44.6% (% = 79 out of 177), 51.0%

Figure 5 | Structural and functional analysis of WT and F383V SerRS. (a) HDX-MS analysis of WT and F383V human SerRS proteins in solution showing that the NLS is conformationally buried in F383V SerRS. The regions associated with major deuterium uptake differences between WT and F383V SerRS are mapped on the primary sequence and on the crystal structure of human SerRS with the same colour coding. The percentage difference of deuterium incorporation indicated under the primary sequence is calculated from the hydrogen–deuterium exchange after 1h for F383V mutant relative to WT SerRS. Deuterium uptake time-course curves for representative peptides are shown on the right. (b) Cell fractionation experiment showing that substitution of D378 with S or R rescues the nuclear localization deficiency of F383V SerRS. (c) Aminoacylation and ATP-PPi exchange assays showing that the NLS is dispensable for aminoacylation and the F383V mutant remains partially active. The partial activity of F383V SerRS is completely lost when the NLS is deleted or released by the second mutation D378R. Error bars represent the s.e.m of triplicate experiments.

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and 45.1% (n = 69 out of 153) of SerRS morphants injected with Δ482-514, NLSmut and F383V SerRS mRNA, respectively. These results strongly suggested that the NLS introduced by UNE-S is essential for vascular development.

To further establish this conclusion, the rationally designed second-site revertant that restores nuclear localization by a compensatory substitution distal to the NLS was tested. Strikingly, the double mutant F383V/D378R SerRS mRNA rescued the abnormal ISV branching defects (8.5%; n = 13 out of 153) with efficiency comparable to that of the WT mRNA (Fig. 6c and Table 1). The result further demonstrated the critical link of the NLS and UNE-S with vasculature development.

The critical role of the NLS in the UNE-S domain indicates a specific function for SerRS in the nucleus of vertebrate cells. Fukui et al. reported that the ko095 SerRS mutant zebrafish had increased expression of vegfa, which was suppressed by microinjection of the aminoacylation-defective T429A SerRS mRNA, suggesting that the noncanonical role of SerRS is related to negative regulation of vegfa expression. Consistently, Herzog et al. showed that inhibition of VEGF signalling rescued aberrant dilatation of the aortic arch vessels in SerRS mutant zebrafish. To address the question of whether the function of SerRS in regulating VEGFA was also linked to UNE-S, we examined vegfa mRNA levels in 72-hpf zebrafish embryos injected with SerRS-MO alone or SerRS-MO together with WT or mutant human SerRS mRNAs. Relative vegfa mRNA levels represented as fold increase (mean ± s.e.m., n = 3, *P < 0.05 (Student’s t-test)) over uninjected control samples, after normalization to the levels of β-actin mRNA. Scale bars in this figure correspond to 100 µm.

Figure 6 | Rescue experiments in zebrafish demonstrating the role of UNE-S in vascular development. (a) Illustration of the phenotype rescue experiment in zebrafish. The SerRS morpholino (SerRS-MO, ~5 ng per embryo) was injected into the yolk of zebrafish embryos (1- to 2-cell stage) to produce a model of intersegmental vessel (ISV) abnormality. Co-injection of SerRS-MO with the WT human SerRS mRNA (~250 pg per embryo) rescued the aberrant ISV branching. The ISV development is recorded at 72h post fertilization and the abnormal ISV branches are indicated by red arrows. (b) Co-injection of SerRS-MO with the NLS-deleted Δ482-514 or NLSmut human SerRS mRNA did not rescue the aberrant ISV branching. (c) Co-injection of SerRS-MO with F383V human SerRS mRNA did not, whereas with F383V/D378R SerRS mRNA did, rescue the aberrant ISV branching. (d) The effect of SerRS nuclear localization on VEGFA transcription. Semiquantitative RT–PCR analysis to evaluate vegfa mRNA levels in 72-hpf zebrafish embryos injected with SerRS-MO alone or SerRS-MO together with WT or mutant human SerRS mRNAs. Relative vegfa mRNA levels represented as fold increase (mean ± s.e.m., n = 3, *P < 0.05 (Student’s t-test)) over uninjected control samples, after normalization to the levels of β-actin mRNA. Scale bars in this figure correspond to 100 µm.
when WT or F383V/D378R SerRS mRNA was co-injected with the SerRS MO (Fig. 6d). In contrast, co-injection of nuclear localization-defective ΔA482-514 or NLSmut or F383V SerRS did not suppress vegfa expression. Thus, regulation of vegfa expression was also dependent on UNE-S and the nuclear localization of SerRS. This observation suggested that the function of SerRS in the nucleus is related to VEGFA regulation.

Discussion

Blood vessel formation, or vasculogenesis, is one of the hallmarks of vertebrate development. During the invertebrate-to-vertebrate transition, the open circulatory systems, where blood is pumped by the heart into the body cavities, of invertebrates evolved into the advanced closed circulatory systems, where vessels are developed and transported to carry the blood to nourish organs and tissues. As the invertebrate-to-vertebrate transition coincides with the appearance of UNE-S, and because UNE-S is shown here to be essential for vascular development, acquisition of UNE-S may have a role in the establishment of the closed circulatory systems of vertebrates.

It is worth noting that, as the tree of life ascended from bacteria to lower and higher eukaryotes, new domains or motifs were added to aaRSs in a progressive and accretive manner. In the case of SerRSs, the acquisition consists of extensions beyond the C-termini of the bacterial orthologs (Fig. 1 and Supplementary Fig. S1). SerRSs from basal eukaryotes have short C-terminal extensions that, as shown for Saccharomyces cerevisiae, while appearing to be important for stability of the synthetase in vitro and in vivo, are dispensable for aminoacylation. However, in going through the transition from invertebrates to vertebrates, the C-terminus of SerRS was significantly expanded into the UNE-S domain. While it remains not important for aminoacylation (Fig. 5c), the expansion included the appearance of a robust NLS.

Our work showed that, because of the NLS brought in by UNE-S, this novel appended domain can mobilize SerRS for translocation from the cytosol to the nucleus—a function that is orthogonal to aminoacylation. Once in the nucleus, SerRS has the additional function of regulating expression of VEGFA, a key regulator of vascular development, most likely at the level of transcription. This nuclear activity of SerRS is in some ways reminiscent of the reported activity of LysRS, which regulates transcription factors MITF and USF2 that are associated with angiogenesis. Once in the nucleus, SerRS has the additional function of regulating expression of VEGFA, a key regulator of vascular development, most likely at the level of transcription. This nuclear activity of SerRS is in some ways reminiscent of the reported activity of LysRS, which regulates transcription factors MITF and USF2 that are associated with angiogenesis.

A lysine-rich peptide derived from a putative NLS sequence at the N-terminus of Lupinus luteus GlnRS was shown to interact with DNA and was suggested to potentially regulate gene expression. The NLS within the UNE-S domain of SerRS is also lysine-rich and has a flexible conformation, as revealed in our crystal structure of human SerRS (which is the first structure of a vertebrate SerRS). This flexibility was further demonstrated through the UNE-S conformational switch induced by the F383V mutation. As flexible protein motifs are known to adapt by induced fit to specific DNA sequence targets, UNE-S may not only mobilize SerRS for nuclear localization but also facilitate interactions with potential targets in the nucleus.

Given that almost all aaRSs have their own distinct elaborations with new domains, our work provides further motivation to discover and understand the function and significance of other synthetase domain accretions in higher organisms. Among them, the N-terminal WHEP domain of TrpRS is of particular interest, because it also appears during the transition from invertebrates to vertebrates and has been shown to regulate the anti-angiogenic activity of human TrpRS.

Methods

Confocal immunofluorescence microscopy. To detect SerRS subcellular localization, HUVEC cells were seeded on cover slips and grown overnight. After fixing and permeabilization, cells were stained with custom-made rabbit anti-SerRS antibody (1 µg ml\(^{-1}\)) followed by Alexa Fluor 488 (green) goat anti-rabbit IgG (at 1:1000 dilution) and 4,6-diamidino-2-phenylindole (1 µg ml\(^{-1}\)). The photos of the cells were taken with a Bio-Rad (Zeiss) Radiance 2100 Rainbow laser scanning confocal microscope in Z-mode (×60 oil objective).

Nuclear fractionation analysis. Human SerRS gene was cloned from HEK293 T cell by RT–PCR and then inserted into pFLAG-CMV2 vector (Sigma-Aldrich) with an N-terminal flag tag. Mutant SerRS constructs were generated by site-directed mutagenesis and transfected into HEK 293 T cells. Cells were harvested after 24 h, and the cytoplasmic and nuclear fractions were separated and extracted by using NE-PER Nuclear and Cytoplasmic Extraction Kit (Thermo Scientific). Exogenously expressed SerRS proteins were detected by western blot analysis using anti-flag polyclonal antibody (Sigma-Aldrich).

Protein overexpression and crystallization. Human SerRS gene was also cloned into PET20b vector (Novagen) with a C-terminal 6×His tag and overexpressed in E. coli BL21 (DE3) cells. SerRS protein was purified in tandem by Ni-NTA affinity (Qiagen) and HiLoad 26/10 Phenyl Sepharose High Performance columns (GE Healthcare). The purified protein was stored in 25 mM Tris–Cl pH 8.0, 0.1 M ammonium sulphate before setting up for high-throughput crystallization using Mosquito liquid transfer robot (TPP Labtech). By use of siting-dropping vapour diffusion method, crystals were obtained at room temperature by mixing 0.1 µl human SerRS (20 mg ml\(^{-1}\)), pre-incipubated with 5 mM ATP and 10 mM MgCl\(_2\) and 0.1 µl reservoir solution (20% PEG 3350, 0.2 M tri-sodium citrate pH 7.0) equilibrated against 70 µl reservoir solution.

Crystal data collection and structure determination. Crystals were cryoprotected with 15% glycerol added to the reservoir solution and flash frozen with liquid nitrogen. A 2.9 Å resolution data set was collected at 100 K on beamline BL-7-1 at Stanford Synchrotron Radiation Laboratory with an ADSC Q315 detector. The crystal belongs to space group P2\(_1\)2\(_1\)2\(_1\) with unit cell dimensions a = 116.80 Å, b = 189.42 Å and c = 230.59 Å. Diffraction data were processed, integrated and scaled with HKL2000. The structure of human SerRS was solved by molecular replacement using Pyrococcus horkohshii SerRS (PDB 2ZR3)\(^{22}\) structure as search model in program PHASER\(^{23}\) from the CCP4 package\(^{24}\). Three homodimers of SerRS were found in one asymmetric unit with ~55% solvent content\(^{25}\). Iterative model building and refinement were performed using Coot\(^{26}\) and Phenix\(^{27}\) with 83% of the polypeptide chains resolved in the final model with R\(_{work}\) of 20.8% and R\(_{free}\) of 25.7% at 2.9 Å resolution.

HDX and mass spectrometry. SerRS mutant proteins were overexpressed and purified as described for the WT protein, and stored in 25 mM HEPES pH 7.5 and 150 mM NaCl. To initiate the HDX reaction, 5 µl of SerRS protein (WT or F383V mutant) was incubated with 45 µl of DBPS buffer in D\(_2\)O (2.6 µM final concentration) for 0.5, 1, 2, 4, 8, 15, 30, 60, 120 or 240 min, followed by simultaneous quench and proteolysis by addition of protease type XIII solution (three-fold dilution from a saturated solution) in 1.0% formic acid, 40 mM TCEP and 4 M urea\(^{28}\). The digested peptide fragments were separated by a fast LC gradient through a ProZap C\(_{18}\) column (Grace Davison, 1.5 µm, 500 Å, 2 × 10 mm) to minimize back exchange\(^{29}\). A post-column splitter reduced the LC eluent flow.
rate to –400–500 nmol min⁻¹ for efficient microelectrospray ionization (micro-ESI)²⁵. Microelectrosprayed HDX samples were directed to a custom-built hybrid linear trap quadrupole 14.5-Tesla FT-ICR mass spectrometer (ThermoFisher, San Jose, CA) for accurate mass measurement. The total data acquisition period for each sample was 6 min. All experiments were performed in triplicate. Data were analysed with a custom analysis package²⁶ and a Python program²⁷. Time-course deuterium incorporation levels were generated by an MEM fitting method²⁸.

Active site titration assay. Active site titration assay was performed in 100 mM HEPES pH 7.5, 20 mM KCl, 10 mM MgCl₂, 50 µM ATP, 22.2 nM γ⁻³²P⁻ATP, 20 mM L-serine, 2 µM γ⁻³²P⁻pyrophosphatase (Roche) and 2 mM dithiothreitol (DTT) as previously described²⁹ to determine the concentration of active enzymes.

ATP-PPi exchange assay. ATP-PPi exchange assay was performed with 0.5 µM SerRS (WT or mutants) in 100 mM HEPES pH 7.5, 20 mM KCl, 10 mM MgCl₂, 2 mM ATP, 500 µM L-serine, 1 mM Na-PPi and 0.07 mM Na⁻³²P⁻Pi and 2 mM DTT as described. The kinetics parameters of ATP and serine for each enzyme (WT at 65 nM; F383V at 95 °C) were determined by varying the ATP and serine concentrations, respectively, while saturating the concentration of the other substrate.

In vitro transcription and purification of RNA. The RNA²⁷(AGA) coding sequence was cloned into pUC18 vector with a T7 promoter sequence at the 5' end and a BstNI restriction enzyme site at the 3' end to allow generation of a linear template that would give the correct 3'–CCA end in the transcript. In vitro transcription was performed in 40 mM Tris–Cl pH 8.0, 25 mM NaCl, 20 mM MgCl₂, 5 mM spermidine, 250 µM γ⁻³²P⁻pyrophosphate, 0.1 µl poly r(C) bovine serum albumin, 5 mM DTT, NTPS with T7 polymerase and BstNI-linearized DNA template at 37 °C. The tRNA transcript was purified by Mono Q HRS/5 anion exchange column (GE Healthcare). The purified tRNA was annealed by heating up to 95 °C and then slowly cooled to room temperature.

Aminocaylase assay. Aminocaylase assays were performed as described with 50–500 nM enzyme in 50 mM HEPES pH 7.5, 20 mM KCl, 10 mM MgCl₂, 4 mM ATP, 2 µM [³²P]L-serine, 20 µM L-serine, 2 mM DTT, 0.1 mg ml⁻¹ bovine serum albumin, 4 µM γ⁻³²P⁻pyrophosphatase (Roche) and 1 µM tRNA⁻³²P⁻transcript. Kinetics parameters of tRNA were determined by varying the tRNA concentration from 0.1x to 10x Kᵥ for each enzyme.

In vivo studies in zebrafish. Transgenic Tg (Flhla: EGFP) fish were maintained at 28.5 °C under continuous water flow and filtration with automatic control for efficient microelectrospray ionization (MEM) fitting method. The CCP4 suite: programs for protein crystallography.

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**Author contributions**

X.X., Y.S., S.K. and X.-L.Y. designed the research; X.X., Y.S. and H.-M.Z. performed the research; X.X., Y.S., H.-M.Z., A.G.M., S.K. and X.-L.Y. analysed the data, X.X., Y.S., H.-M.Z., E.C.S., A.G.M., M.G., S.K. and X.-L.Y. wrote the paper.

**Additional information**

Accession codes: The atomic coordinates for the SerRS crystal structure have been deposited in the Protein Data Bank under accession code 3VBB.

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