Supplementary Methods

1. Differential Expression Analysis

Gene expression data from GSE85358 were obtained from the GEO database using the GEOquery package in R. Data were normalized using the normalizeBetweenArrays function from the limma package. Differentially expressed genes (DEGs) between young and aged samples were identified using linear models. Genes with an adjusted P-value < 0.05 and |log2 fold change| > 0.25 were considered significant.

2. Weighted Gene Co-expression Network Analysis (WGCNA)

WGCNA was performed using the WGCNA R package. The expression matrix was transposed so that samples were in rows and genes in columns. A soft-thresholding power of 7 was selected to approximate scale-free topology. The minimum module size was set to 130, and modules were merged using a cut height of 0.25. Module–trait relationships were evaluated using Pearson correlation with aging phenotype.

3. Machine Learning for Feature Selection

- LASSO regression was performed using the glmnet package with 5-fold cross-validation to determine the optimal lambda.
- Random forest analysis was conducted using 500 trees, and feature importance was evaluated based on MeanDecreaseGini.
- SVM-RFE was implemented using a custom workflow based on the e1071 package with 5-fold cross-validation. The optimal feature number was selected based on the minimum classification error.

4. Molecular Docking

Molecular docking was performed using the CB-Dock2 platform, which automatically identifies potential binding cavities. Docking simulations were carried out using AutoDock Vina (version 1.2.3). For each protein–ligand pair, three independent docking runs were performed. Binding poses with RMSD < 2.0 Å were considered reliable, and the best binding energy was recorded.

5. Molecular Dynamics Simulation

Molecular dynamics simulations were performed using GROMACS. The protein was parameterized using the amber99sb-ildn force field, and the ligand was parameterized using the GAFF force field via

ACPYPE. The system was solvated using the TIP3P water model in a cubic box with a 1.0 nm buffer distance. Counter ions were added to neutralize the system. Energy minimization was followed by NVT and NPT equilibration (100 ps each). Production simulations were conducted for 20–100 ns. Trajectory analyses included RMSD, RMSF, radius of gyration (Rg), solvent accessible surface area (SASA), hydrogen bonding, and free energy landscape (FEL).