

Supplementary Materials

Structural and Biochemical Analysis of Human Schlafen5 Reveals the Molecular Basis of Its Anti-Tumor Function

Jin-Yu Yang^{1,2,*}, Jun-Ying Ou^{1,†}, Meng Luo^{1,†}, Zi-Wen Wang^{1,†}, Yong-Qiang Zheng¹, Ze-Xian Liu¹, and Song Gao^{1,3,4,*}

¹ State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-sen University Cancer Center, Guangzhou 510060, China

² Biologics Discovery Department, BeOne (Guangzhou) Innovation Technology Co., Ltd., Guangzhou 510005, China

³ Integrated Traditional Chinese and Western Medicine Research Center, Sun Yat-sen University Cancer Center, Guangzhou 510060, China

⁴ Department of Experimental Research, Sun Yat-sen University Cancer Center Gansu Hospital, Lanzhou 730000, China

* Correspondence: jinyuyang1991@163.com (J.-Y.Y.); aosong@sysucc.org.cn (S.G.)

† These authors contributed equally to this work.

Received: 2 December 2025; Revised: 25 February 2026; Accepted: 12 July 2026; Published: 10 September 2026

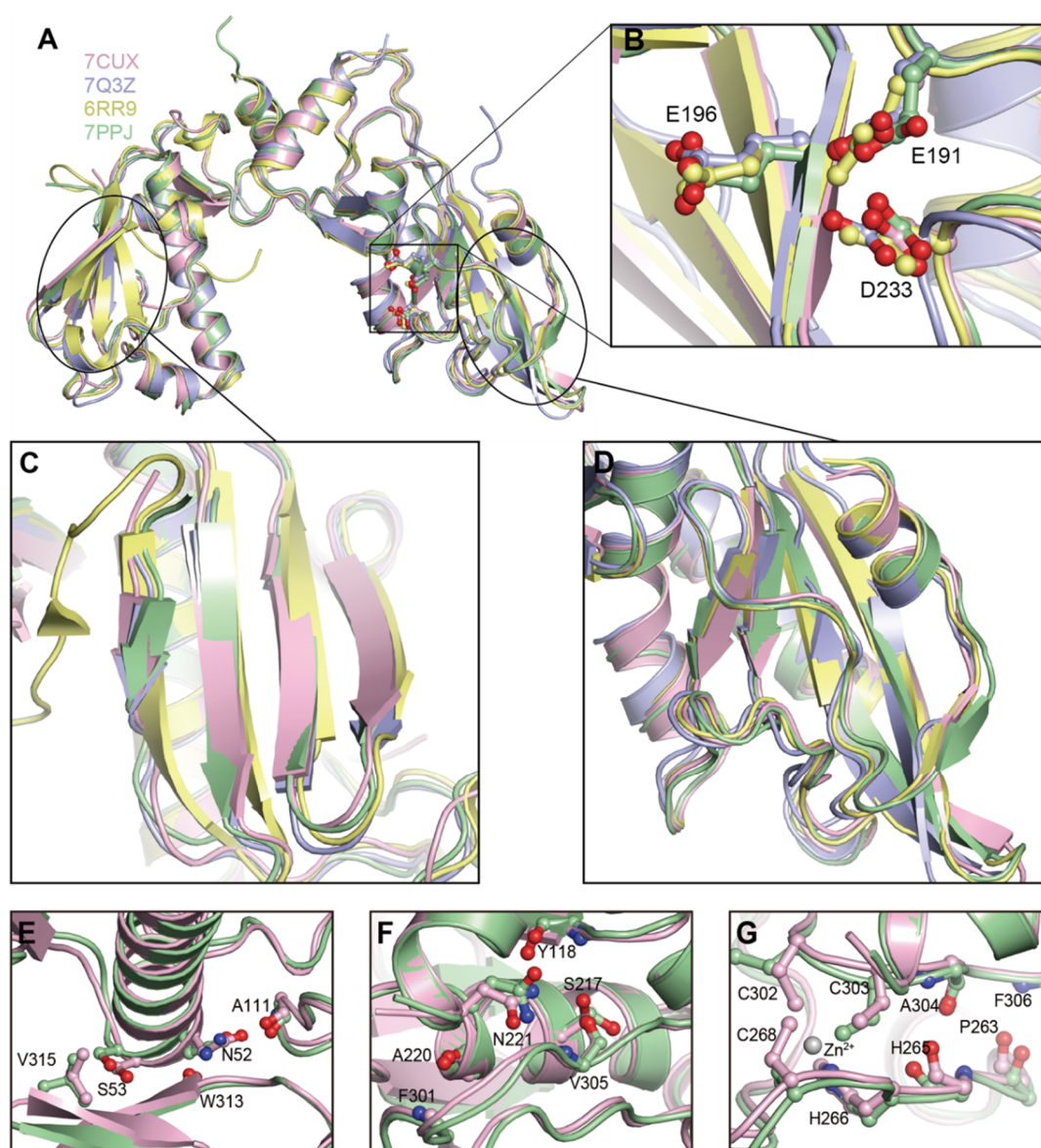


Figure S1. Superimposition of four SLFN5-N structures. (A) Crystal structure of hSLFN5-N (PDB ID: 7CUX) determined by our team were compared with another crystal structure of hSLFN5-N (7Q3Z, 6RR9) and a



Copyright: © 2025 by the authors. This is an open access article under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Publisher's Note: Scilight stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

hSLFN5-N structure from the full-length SLFN5 structure (7PPJ) determined by electron microscopy. The r.m.s.d between 7CUX with 7Q3Z, 6RR9 and 7PPJ are 1.0 Å, 0.88 Å and 0.92 Å, respectively. **(B)** Overlay of the catalytic modules. **(C,D)** Local structural comparison in N-lobe **(C)** and C-lobe **(D)**. **(E–G)** Comparison between the cryo-EM structure (7PPJ) and the crystal structure (7CUX) of hSLFN5-N. Residues mediating the interactions between the N-lobe and BD **(E)**, between the C-lobe and BD **(F)**, and within the CCCH-type zinc finger **(G)** were shown.

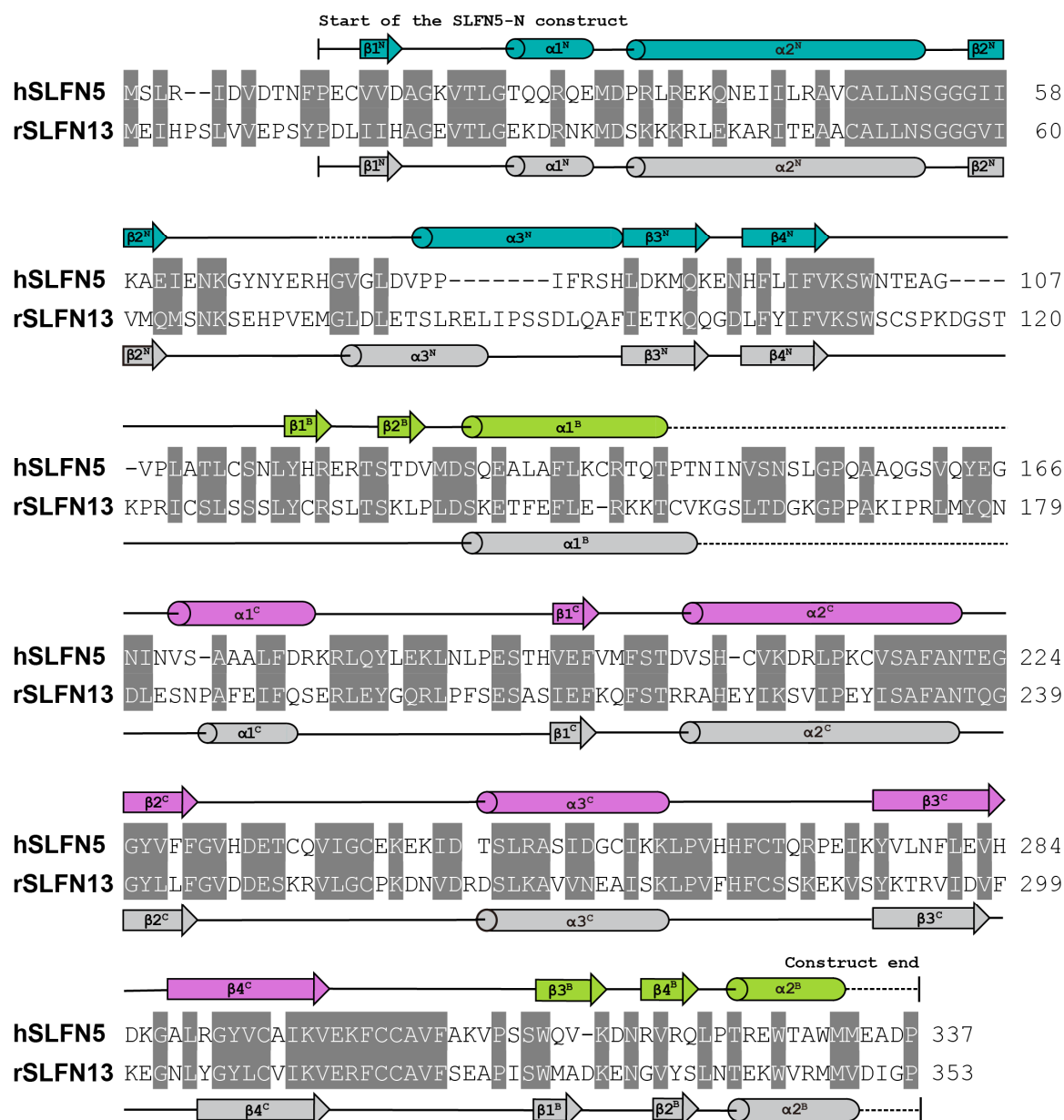


Figure S2. Sequence alignment between hSLFN5-N and rSLFN13-N. The amino acid sequences of hSLFN5-N (UniProt accession: Q08AF3) and rSLFN13-N (UniProt accession: Q5U311) are aligned using ClustalW2, adapted with permission from Ref. [1]. Residues with a conservation over 80% are in grey shades. α -helices are shown as cylinders and β -strands as arrows for hSLFN5 and rSLFN13. The secondary structure of hSLFN5-N are colored and named as Figure 1B. The secondary structure of rSLFN13-N are colored grey.

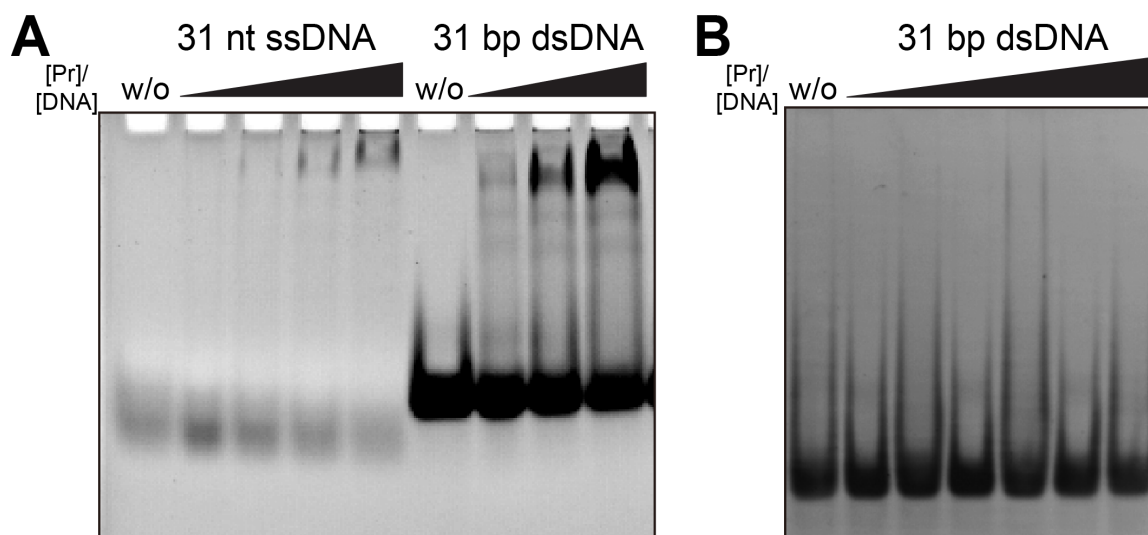


Figure S3. The DNA binding activity of hSLFN5-N and rSLFN13-N. **(A)** EMSA result showing the interaction between hSLFN5-N and ssDNA/dsDNA. hSLFN5-N and DNA of increasing molar ratios (1:1, 2:1, 4:1, 8:1) were incubated for 1 h at room temperature. Pr, protein. **(B)** rSLFN13-N does not interact with dsDNA. rSLFN13-N and dsDNA of increasing molar ratios (4:1, 8:1, 16:1, 24:1, 32:1, 64:1) were incubated for 1 h at room temperature.

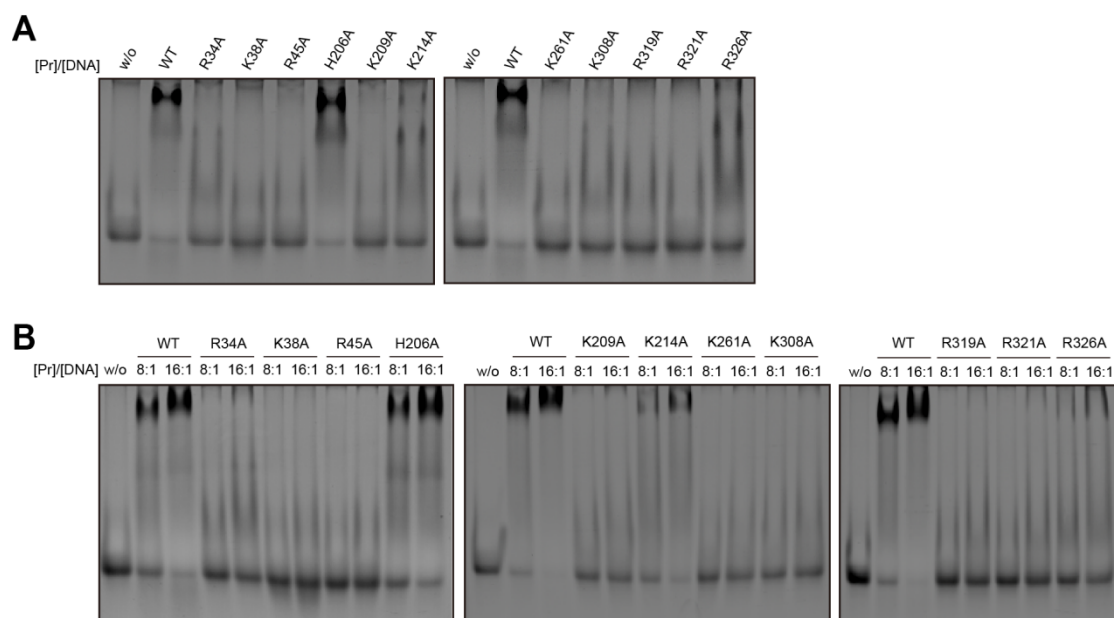


Figure S4. The key amino acids of hSLFN5-N for DNA affinity. **(A)** Wild-type (WT) hSLFN5-N and corresponding mutants were individually incubated with 30 nt random DNA in molar ratio 16:1. **(B)** The DNA binding activity of hSLFN5-N is dependent of the charged amino acids around the central valley. Wild-type (WT) hSLFN5-N and corresponding mutants were individually incubated with 31 bp DNA in molar ratio 8:1 or 16:1.

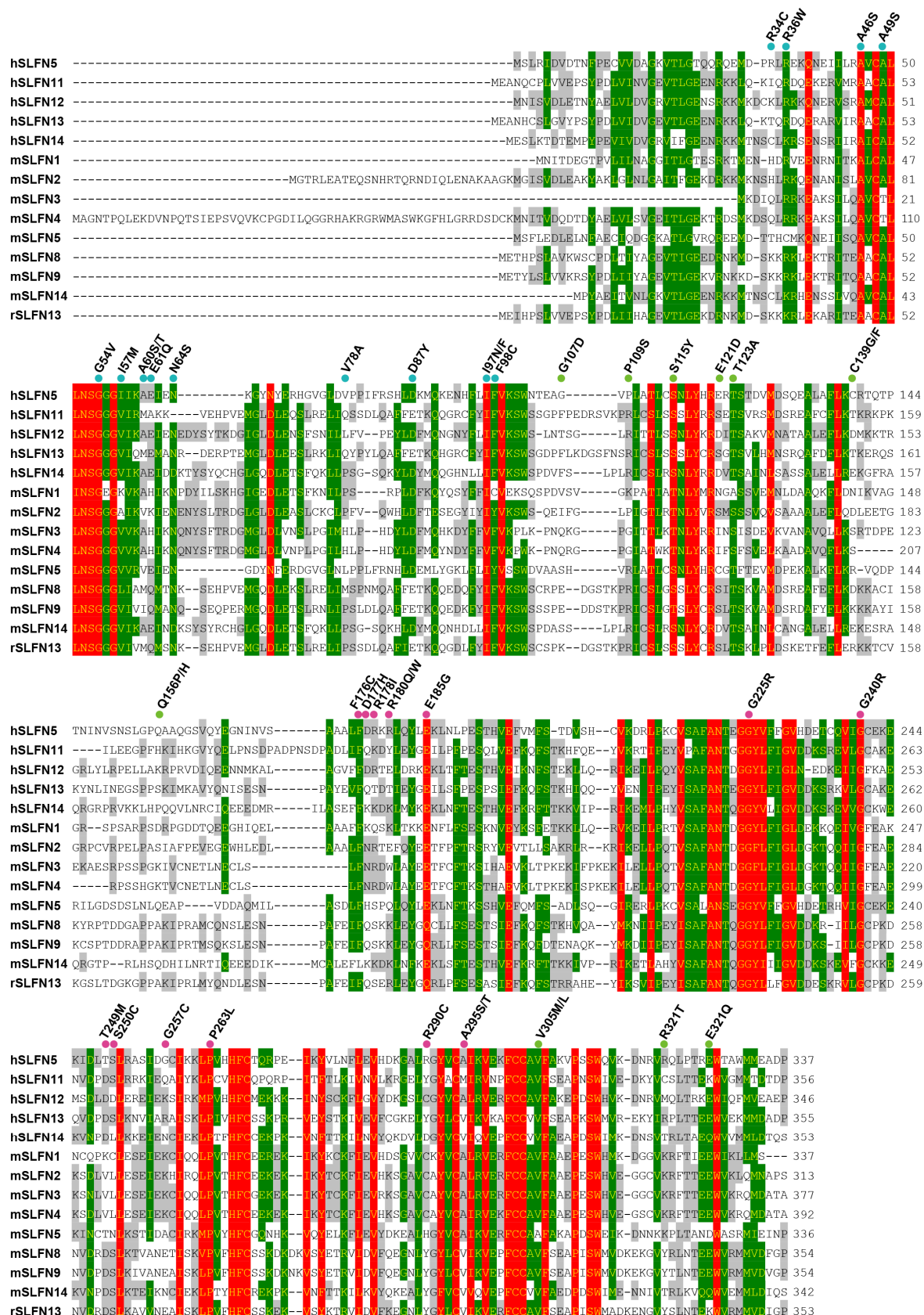


Figure S5. Sequence alignment of the SLFNs-N. Amino acid sequences of human (h)SLFN5 (UniProt accession Q08AF3), hSLFN11 (Q7Z7L1), hSLFN12 (Q8IYM2), hSLFN13 (Q68D06), hSLFN14 (P0C7P3), mouse (m)SLFN1 (Q9Z0I7), mSLFN2 (Q9Z0I6), mSLFN3 (Q9Z0I5), mSLFN4 (Q3UV66), mSLFN5 (Q8CBA2), mSLFN8 (Q7TMF), mSLFN9 (Q7TME9), mSLFN14 (V9GXG1) and rat (r)SLFN13 (Q5U311) are aligned using ClustalW2. Residues with a conservation of 100% are in red shades, greater than 80% in green shades and 60% in grey shades, respectively. The cancer-related mutations of SLFN5 are indicated by dots above the sequence which are colored according to the subdomain they belong to (same color scheme as in Figure 1a).

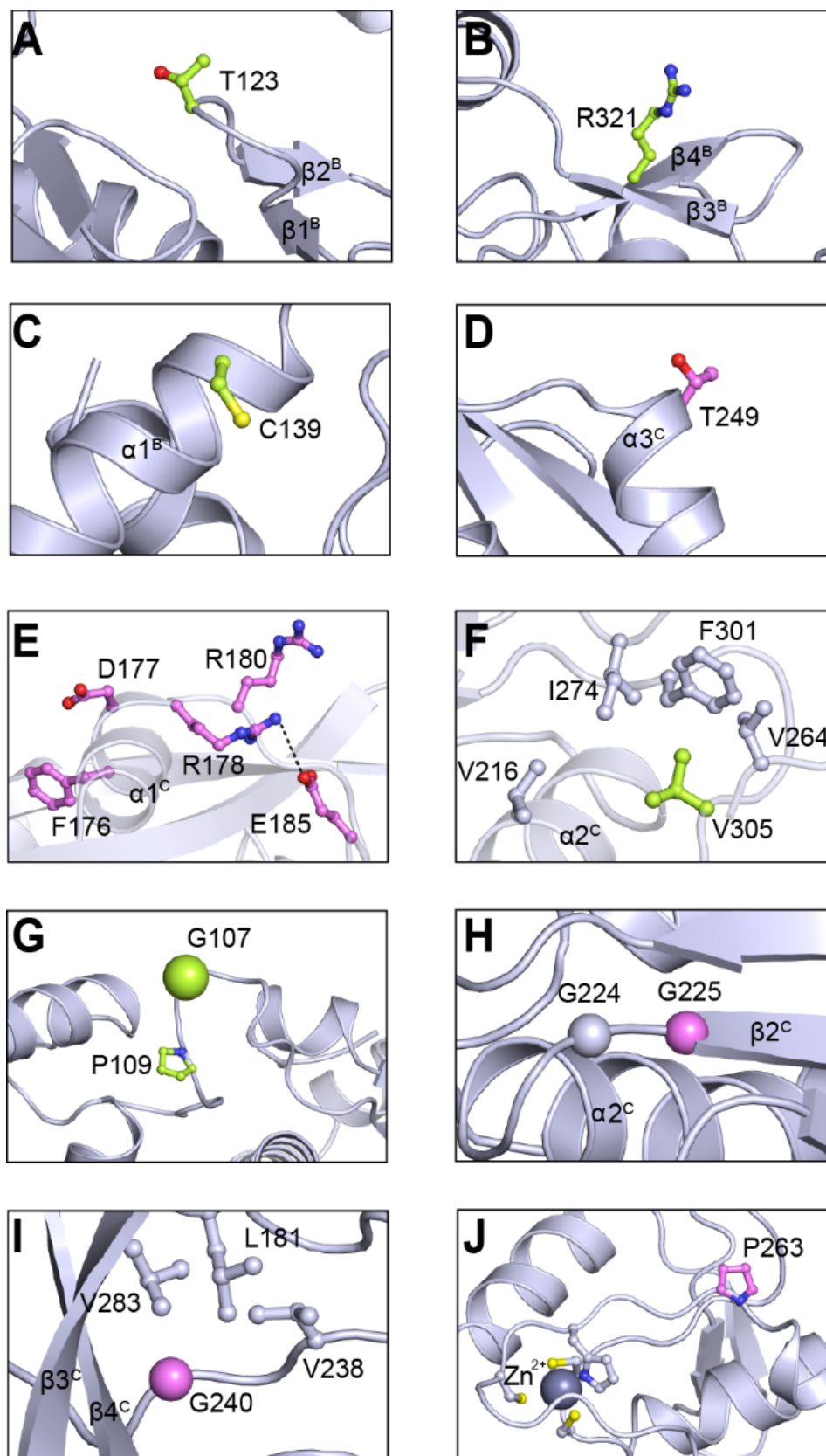


Figure S6. Cancer-related mutations in BD and C-lobe. (A–E) Exposed residues. Thr123 (A), Arg321 (B), C139 (C), Thr249 (D), Phe176, Asp177, Arg178, Arg180 and Glu185 (E). Residues subjected to mutation are colored lemon (from BD) or magenta (from C-lobe), and the surrounding residues are colored gray. Note that Arg178 at the end of $\alpha 1^C$ forms a salt bridge with Glu185. (F) Val305 of BD is surrounded by several hydrophobic residues. (G–J) Glycine and proline mutations. Gly107 and Pro109 (G), Gly225 (H), Gly240 (I), Pro263 (J). Mutations on these residues may alter the flexibility/rigidity of local configuration, and cause folding problems of the protein.

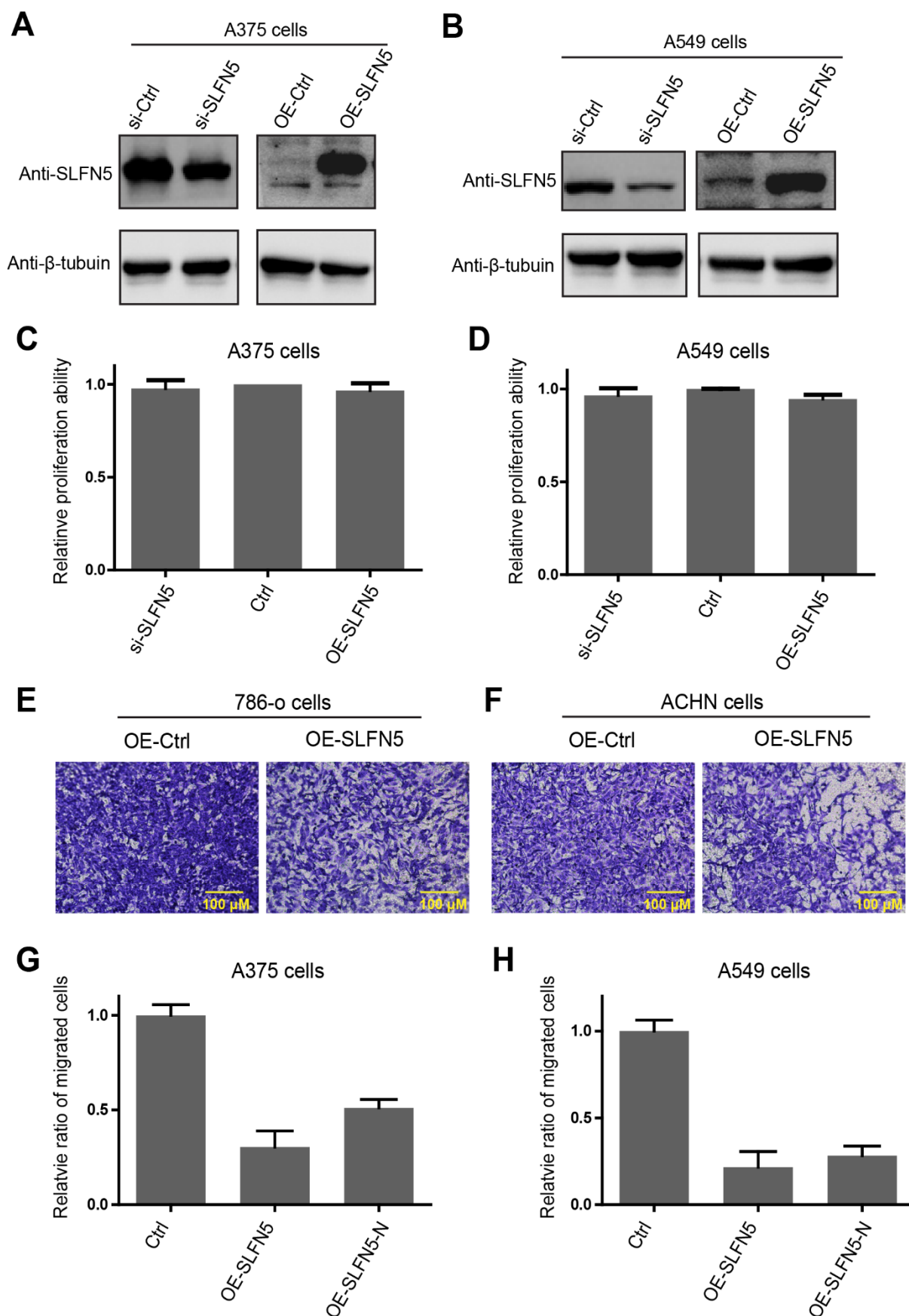


Figure S7. Effects of over-expressed SLFN5 on tumor cells migration. (A,B) The expression of SLFN5 in A375 cells (A) and A549 cells (B) was verified by western blot (WB). A375 cells transiently transfected with control siRNA (si-Ctrl), SLFN5 siRNA (si-SLFN5), control vector (OE-Ctrl) or SLFN5 vector (OE-SLFN5) were collected and lysed, total lysates were resolved by SDS-PAGE and immunoblotted with an anti-SLFN5 antibody, anti-Flag antibody or an anti-β-tubulin antibody, as indicated. (C,D) Proliferation of A375 cells (C) and A549 cells (D). Error

bar indicates s.d. (n = 4). (E,F) Migration of 786-o cells (E) and ACHN cells (F) transfected with OE-Ctrl, or OE-SLFN5. 24 h after transfection, the cells were collected and counted. 5×10^4 cells were seeded into chamber to growth for 20 h. (G,H) Both SLFN5 and SLFN5-N inhibited the cell migration in A375 cells (G) and A549 cells (H). SLFN5-N was just slightly weaker than SLFN5 on inhibitory effect. Error bar indicates s.d. (n = 4).

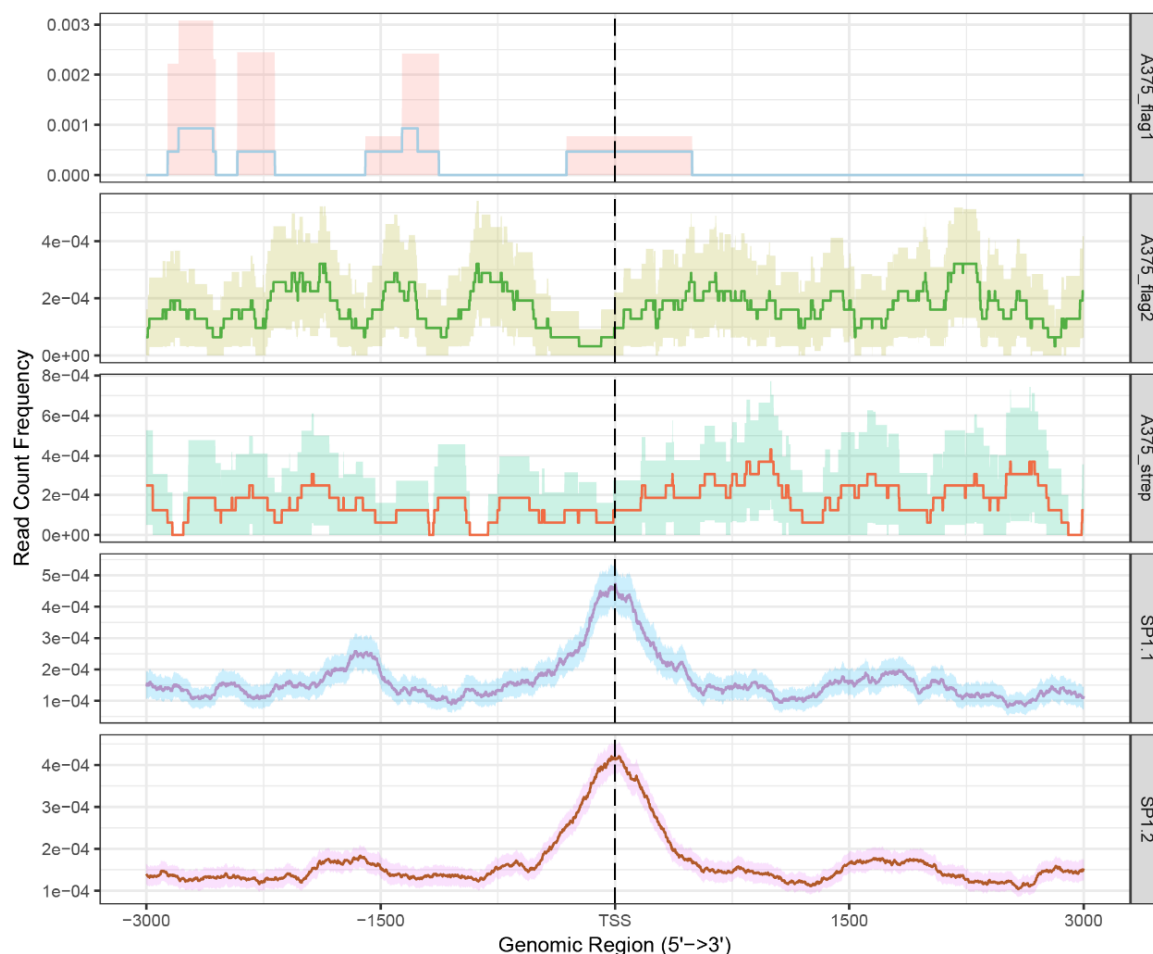


Figure S8. ChIP-Seq results of SLFN5 in A375 cells. A375_flag1, results from pLVX-Flag-SLFN5 expressing cells. A375_flag2, results from pLVX-SFB-SLFN5 expressing cells. A375_strep, results from pLVX-SFB-SLFN5 expressing cells. SP1.1 (GSM1661547) and SP1.2 (GSM1661546), validated transcription factors in A375 cells as positive controls.

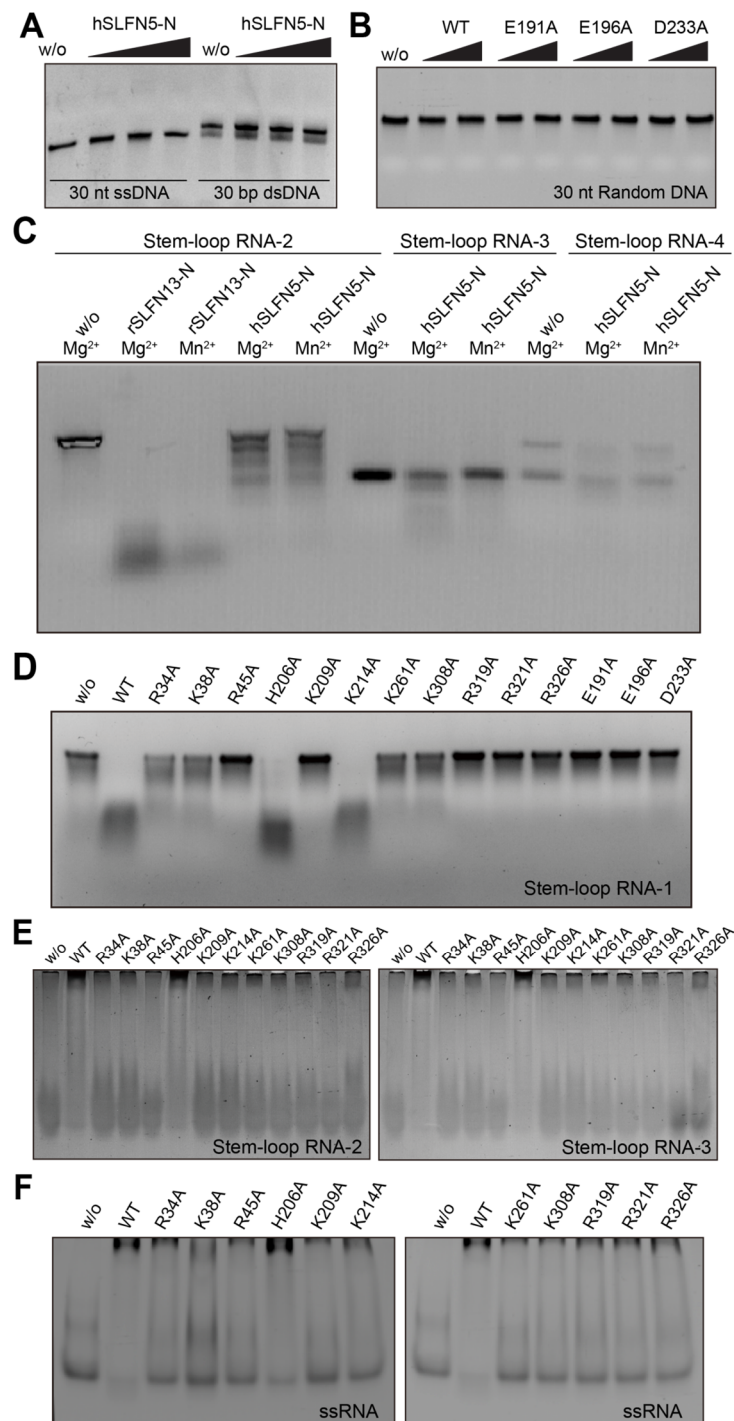


Figure S9. The nucleic acid substrates processing ability of hSLFN5-N. (A,B) hSLFN5-N does not cleave DNAs. hSLFN5-N and mutants of increasing concentrations (2, 4 μ M) were individually incubated with 30-nt ssDNA, 30-nt dsDNA (A) and 30-nt random DNA (B). (C) hSLFN5-N cleaves stem-loop RNAs. 2 μ M hSLFN5-N was individually incubated with transcribed stem-loop RNAs for 30 min at 37 °C in the presence of either Mg^{2+} or Mn^{2+} . hSLFN13-N was used as a positive control. (D) Stem-loop RNA-1 cleavage by hSLFN5-N and mutants. 1 μ M wild-type hSLFN5-N and mutants were individually incubated with stem-loop RNA-1 for 30 min at 37 °C. (E,F) The RNA binding activity of hSLFN5-N and related mutants. Wild-type (WT) hSLFN5-N and corresponding mutants were individually incubated with stem-loop RNA-2, stem-loop RNA-3 (E) or 30-nt ssRNA (F) molecules. The molar ratio of protein and DNA/RNA is 8:1.

Reference

1. Thompson, J.D.; Higgins, D.G.; Gibson, T.J. CLUSTAL W: Improving the Sensitivity of Progressive Multiple Sequence Alignment through Sequence Weighting, Position-Specific Gap Penalties and Weight Matrix Choice. *Nucleic Acids Res.* **1994**, *22*, 4673–4680