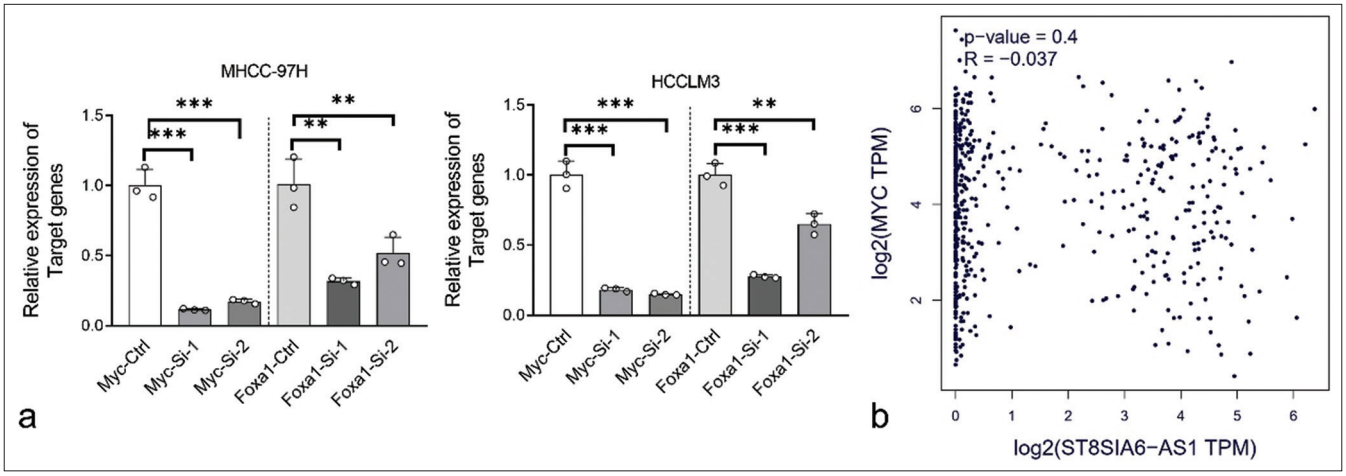

Supplementary Figure 3: (a) Small interfering RNA was used to knock down MYC and Forkhead box A1 (FOXA1) in MHCC-97H and HCCLM3, respectively. The expression level of MYC and FOXA1 was detected by quantitative polymerase chain reaction. (b) Co-expression analysis between ST8SIA6 antisense RNA 1 and MYC in liver tumors and GETx liver tissues (data from GEPIA). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Supplementary Methods

Colony Formation Assay

Digest the cells with trypsin, centrifuge to remove the supernatant, resuspend the cell pellets in fresh culture medium, and count the cells. Adjust the cell concentration to 10,000 cells/mL using a gradient dilution method. Seed 1,000 cells (100 μL) per well in a six-well plate, and add culture medium to a final volume of 3 mL (DMEM + 10% FBS + antibiotics [Yuanpei: S120JV, working concentration: 10 units/mL penicillin, 10 μg/mL streptomycin, and 0.025 μg/mL amphotericin B]). Shake well to ensure even distribution of cells in the culture medium, and incubate in a cell culture incubator. Replace the culture medium with fresh pre-warmed medium every 4 days, and culture for a total of 2 weeks. After 2 weeks, discard the culture medium, add 1 mL PBS to wash away residual medium, then add 600 μL of 4% paraformaldehyde solution to fix the cells. After 10 min, discard the paraformaldehyde and wash twice with PBS to remove residual paraformaldehyde. Add 600 μL of crystal violet staining solution (Beyotime: C0121), stain for 20 min, discard the staining solution, and gently rinse with water to remove residual stain. Air dry at room temperature and take photographs.

Scratch Assay

Seed the cells to be tested in a 6-well plate and incubate in a cell culture incubator until they reach 90% confluence. Use a 200 μL pipette tip to create a scratch, ensuring the scratch is straight, with uniform force and width. After scratching, replace the medium with fresh pre-warmed medium to wash away detached cells, and take photographs under a microscope (0 h), marking the photographed positions. Take photographs at the marked positions at 24 h, 48 h, and 72 h after the initial scratch. Analyze the images to compare the scratch healing rates among different groups.

Transwell Assay

Digest the experimental cells with trypsin, centrifuge to remove the supernatant, resuspend the cell pellets in DMEM, and count the cells. Adjust the cell concentration to 1×10^6 cells/mL. Add 800 μL of culture medium (DMEM + 10% FBS) to a 24-well plate, insert the Transwell chamber, and add 200 μL of the cell suspension from step 1 to the chamber. Incubate in a cell culture incubator for 48 h. After 48 h, discard the medium in the wells, use a cotton swab to remove the cells inside the chamber, and wash away detached cells with 500 μL PBS. Add 500 μL of 4% paraformaldehyde to fix the cells. After 10 min, discard the paraformaldehyde and wash twice with 500 μL PBS.

Add 300 μL of crystal violet staining solution to the chamber, stain for 20 min, and gently rinse the chamber with water to

remove residual stain. Air dry at room temperature, and take photographs under a microscope to observe and assess the number of cells that have migrated to the outer side of the chamber.

Co-Immunoprecipitation (Co-IP) Assay

Digest the cells (overexpressing HA-tagged C-Myc and Flag-tagged FOXA1) with trypsin, centrifuge to collect the cells, and count them. Wash the cells twice with PBS to remove residual serum. Resuspend 1×10^7 cells in 700 μL of weak RIPA buffer (Beyotime, P0013D) and add 7 μL of protease inhibitor (Beyotime, P1005). Place on ice for 10 min and sonicate in a pre-cooled ultrasonic processor for 10 cycles (each cycle: 1 min, 30 s sonication, 30 s pause).

After sonication, centrifuge at 15,000 g for 10 min in a pre-cooled centrifuge. Transfer the supernatant to two new 1.5 mL centrifuge tubes, with approximately 300 μL of lysate in each tube. Reserve about 80 μL as input protein and store at 4°C. Add anti-HA antibody (or anti-Flag antibody) or non-specific IgG antibody as a negative control to the two 1.5 mL centrifuge tubes. Incubate overnight at 4°C with rotation. After overnight incubation, add 20 μL of Protein A/G magnetic beads to each tube to bind the antibodies in the lysate. Incubate at 4°C with rotation for 2 h. After incubation, place the 1.5 mL centrifuge tubes on a magnetic rack. After approximately 3 min, discard the supernatant once the beads have aggregated on the tube wall. Remove from the magnetic rack and add 1 mL of pre-cooled PBS. Incubate at 4°C with rotation for 5 min. Place back on the magnetic rack and discard the PBS once the beads have aggregated. Repeat this washing step 3 times, replacing the PBS with 500 μL of pre-cooled weak RIPA buffer on the third wash. Add 40 μL of weak RIPA buffer to the bead pellet and 10 μL of 5×SDS loading buffer (also add 20 μL of 5×SDS loading buffer to the 80 μL input protein lysate). Heat at 100°C in a metal bath for 10 min, then store at -20°C. Analyze the Co-IP samples by Western blotting.

Dual-Luciferase Reporter Assay

Cloning of the Fragment: Based on database predictions, we cloned the DNA fragment from −260 bp to +1489 bp of the ST8SIA6-AS1 transcription start site. A: Extraction of Genomic DNA: Extract genomic DNA from HepG2 cells (Beyotime: D0061). B: Amplification of the Fragment: Use reagents from Vazyme (DNA polymerase: Phanta Max Master Mix, P515) and primers designed to complement the 5' and 3' ends of the −260 bp to +1489 bp fragment, with recombination arms matching the sequences near the restriction sites of the pGL3-basic plasmid. Perform PCR according to the reagent instructions. Loading the Fragment onto the pGL3-basic Vector: First, linearize the pGL3-basic vector by restriction digestion, then use DNA recombinase to insert the DNA fragment into the linearized plasmid. Transfection: Co-transfect 293T cells with the pGL3-basic plasmid containing the -260 bp to +1489 bp fragment, along with overexpression plasmids for C-MYC, FOXA1, and an empty vector. After 24 h, measure the luciferase activity of the cells. Measurement of Luciferase Activity: Use the Dual-Glo® Luciferase Assay System (Promega) to lyse the cells and measure luciferase activity according to the reagent instructions. Firefly luciferase and Renilla luciferase serve as the experimental and internal control values, respectively. Calculation: Determine the binding and regulatory effects of MYC and FOXA1 proteins on the ST8SIA6-AS1 promoter region by calculating the ratio of experimental to internal control values and comparing the differences between groups.