Electronic Supplementary Material (ESI) for Molecular Omics.

KIF2C as a Potential Therapeutic Target: Insights from lung adenocarcinoma Subtype Classification and Functional Experiments

1. Materials and Methods

2.6 siRNA Interference Assays

A549 and H1299 cells were seeded in 6-well plates. The day before the transfection experiment, the cell density of A549 and H1299 cells needed to reach 60%-80%. Six hours before transfection, the incomplete medium was replaced. GenePharma (Shanghai, China) constructed three different small interfering RNA (siRNA) molecules targeting KIF2C (si-KIF2C) as well as their negative control (si-NC). 100 pmol of siRNA was diluted in 250 μl of Opti-MEM (ThermoFisher Scientific, #31985070), and 5 μl of Lipofectamine 2000 (Thermo Fisher Scientific, #11668500) was also diluted in 250 μl of Opti-MEM low-serum medium, mixed gently, and incubated at room temperature for 5 minutes. The diluted siRNA and Lipofectamine 2000 were mixed and left at room temperature for 20 minutes. 500 μl of transfection solution was added to each well, gently mixed. Incubating at 37°C. After 6 hours of transfection, the culture medium was replaced, and gene expression was detected 24 hours later. The sequences of the related siRNAs are as follows: si_NC (5'-3': UUC UCC GAA CGU GUC ACG UTT), si_KIF2C#1 (5'-3': GCU GAU UCC AGG CAA UUU ATT), si_KIF2C#2 (5'-3': GGC CAA GAA AGA AAU UGA UTT), and si_KIF2C#3 (5'-3': CAG GCU AGC AGA CAA AUA ATT).

2.9 Construction of a stable sh-KIF2C LA-4 cell line.

The sh-KIF2C (5'-3') and sh-nc (5'-3') sequences were designed based on the NCBI mouse KIF2C gene sequence (NM_001290662). The interference vector plasmid pLVX-shRNA2 (Fenghui Biotechnology, #BR005) was selected. HEK-293T cells (Pricella) in good growth status were used as the packaging cells. When the cell density reached 60%~80%, the transfection was performed. Six hours before transfection, Opti-DMEM (ThermoFisher Scientific, #31985070) was replaced for culture. pLVX-shRNA2, psPAX2, and pMD2.G plasmids (total 2 μg) were diluted with 400 μl Opti-DMEM, and 8 μl Lipofectamine 2000 (Thermo Fisher Scientific, #11668500) was diluted with 400 μl Opti-DMEM. The solutions were gently mixed, and after 5 minutes, the Lipofectamine 2000 mixture was slowly added to the plasmid mixture and allowed to stand for 10 minutes. The prepared transfection solution was then added to the cell culture dish and gently mixed. After culturing for 6 hours at 37°C with 5% CO2, complete DMEM culture medium was replaced, and the expression of ZsGreen green fluorescent protein in HEK-293T cells was observed under a fluorescence microscope (Leica) 24 hours later to analyze the plasmid transfection efficiency. Forty-eight hours later, the supernatant of the cells was collected, centrifuged, and filtered as the viral solution for subsequent target cell transfection. The collected viral solution was used to transfect LA-4 cells with good growth status and a cell density of 40%-60%. Forty-eight hours later, the expression of green fluorescent protein in LA-4 cells was observed under a fluorescence microscope, followed by selection of non-transfected cells with a predetermined concentration of puromycin (Beyotime, #ST551) until the expression of green fluorescent protein in LA-4 cells reached over 90%, which could be used as the stable LA-4 cell line for subsequent animal experiments.

2.10 Subcutaneous Tumor Formation

10 SPF-grade C57BL/6 mice,age (8-10 weeks), weight (22-24g), were all purchased from Henan SKBS Biotechnology Co., Ltd. of Henan province. All experimental protocols were strictly conducted in accordance with the guidelines approved by the Animal Ethics Committee of the Medical College of Anhui University of Science and Technology (Ethical Approval Number: NO.HX-002). The mice were housed in a controlled environment at the Experimental Animal Center of the Medical College of Anhui University of Science and Technology (temperature 20±2°C, relative humidity 55±5%, 12-hour light/dark cycle). The mice were supplemented with food three times a week, provided with ample drinking water daily, and their body weight was monitored to assess their health status. After a 7-day acclimation to the housing conditions, they were randomly assigned to respective groups to establish animal models.

Ten mice were randomly divided into two groups, and 100ul (PBS) containing 1 × 10^5 sh_NC and sh_KIF2C mouse lung cancer cells (LA-4) were subcutaneously injected into the axillary area of each group of mice (n=5). The health of the mice was monitored twice a day, including observations of tumor growth, body weight, appetite, and behavior.

The entire experimental process lasted for 27 days. Throughout the experiment, strict animal welfare guidelines were followed, and at the end of the experiment, the mice were euthanized by cervical dislocation after inhaling 2% isoflurane (product number R510-22-10; RWD Life Sciences Co., Ltd.). The researchers recorded the volume and weight of the tumors and calculated the tumor volume using the formula V=π/6 × L (length) × W (width) × H (height). Data analysis was performed using GraphPad Prism software (version 9.5), and the tumor volume and weight of the two groups were analyzed using the two-tailed T-test.

2.11 Immunofluorescence Analysis of Marker Proteins

5-micrometer thick sections were prepared from mouse tumor tissue samples that had been embedded in paraffin and fixed with 4% formaldehyde. The sections then underwent deparaffinization. After deparaffinization, the sections were placed in antigen retrieval buffer containing 0.01M pH9.0 Tris-EDTA (Servicebio, #G1207) and heated in a microwave at high power for 5 minutes, followed by cooling for 8 minutes, then reheating at low power for 5 minutes, and cooling again for 8 minutes. This process ensured that the buffer did not evaporate completely or cause the sections to become excessively dry. Once the sections had cooled to room temperature, they were blocked with 5% normal goat serum diluted in 1X TBST (5 ml) containing 250 µl of normal goat serum (Thermo Fisher Scientific, #16210064, Gibco) for 30 minutes. Following blocking, the samples were washed three times for 5 minutes each with 1X TBST on a shaker.

After washing, the sections were fully covered with the primary antibody dilution solution prepared with Albumin Bovine V (Biosharp, #BS114) and incubated overnight at 4°C in a humidified environment. Post-incubation, the samples were warmed to room temperature for 15 minutes, the primary antibody solution was removed, and the sections were washed three

times for 5 minutes each with 1X TBST. The sections then underwent secondary antibody incubation, where they were placed in a fluorescence-labeled secondary antibody dilution solution corresponding to the species of the primary antibody and incubated for 1 hour at 37°C in the dark. After the secondary antibody incubation, the sections were washed three times for 5 minutes each with 1X TBST. All these steps were carried out on a shaker.Finally, the cell nuclei were stained with DAPI dye and observed under a Leica 3000 confocal fluorescence microscope. After observation, the tissue samples were properly stored. The immunofluorescence-stained sections were analyzed using ImageJ software version V1.8.0.112. The primary antibodies used in this experiment included:E-cadherin and vimentin (Cell Signaling Technology, #3195T, #5741T,1:1 000) and KIF2C (Proteintech,#28372-1-AP, 1:1000).

2. Supplementary Figures

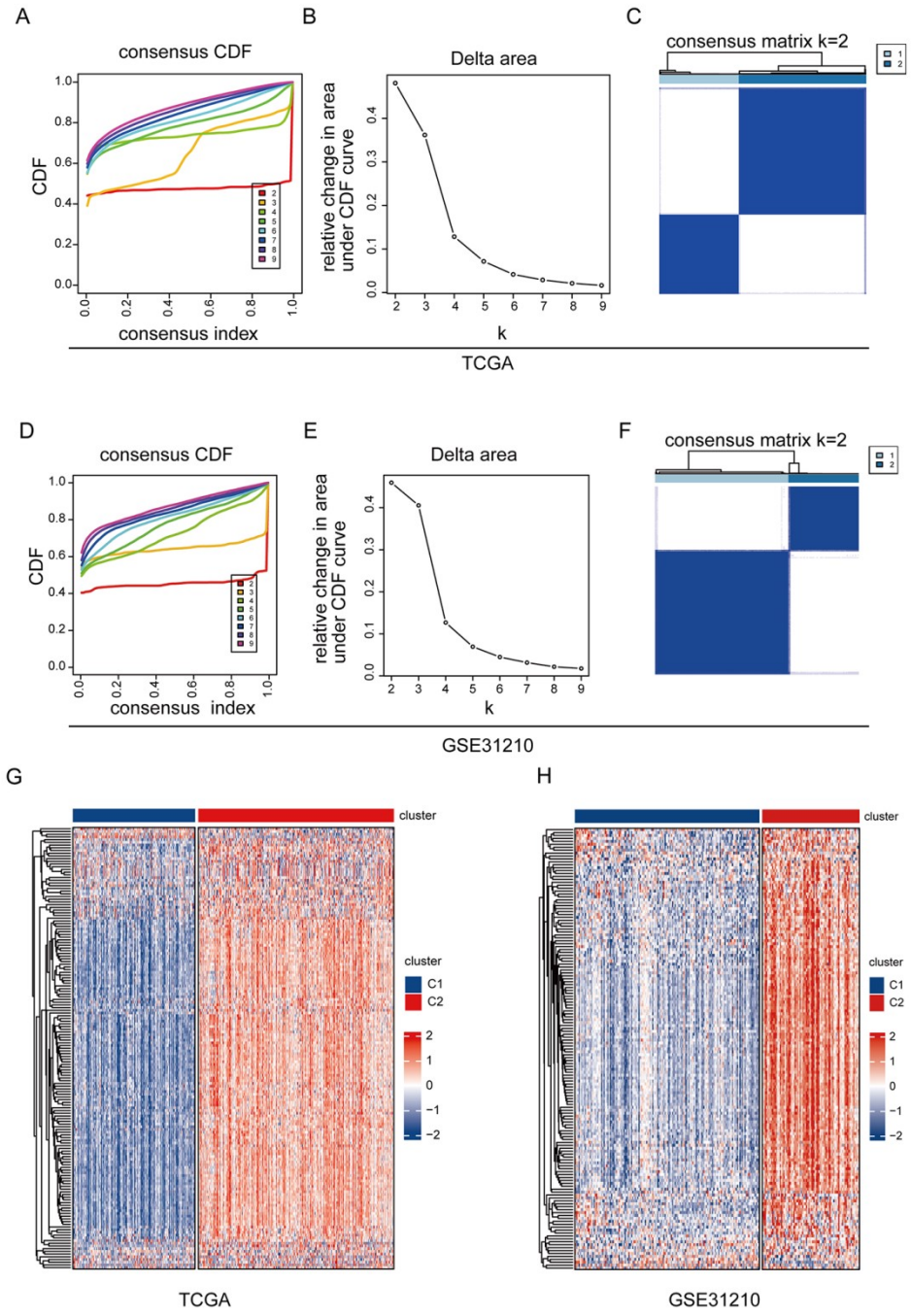

Figure S1. Prognosis-related genes were assigned to tumor risk classification in the TCGA and GEO cohorts. (A) Cumulative distribution function (CDF) of consensus clustering. (B) Relative changes in the CDF area under the curve (CDF Delta area). (C) Consistency between clustering results in the heatmap. (D) Cumulative distribution function (CDF) of consensus clustering. (E) Relative changes in the CDF area under the curve (CDF Delta area). (F) Consistency between clustering results in the heatmap.(G-H) Heatmap of gene expression in different subgroups.

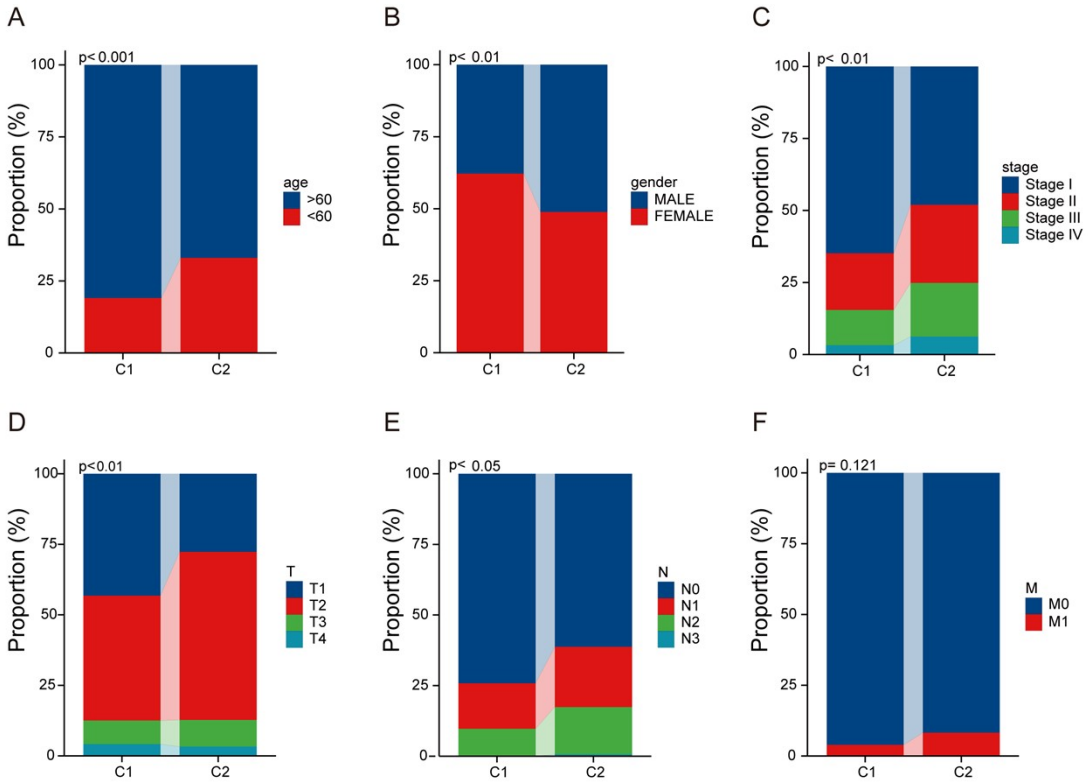

Figure S2. Comparison of clinical characteristics between C1 and C2 subtypes. The differences in (A) age, (B) gender, (C) Stage, (D) T: tumor size stage, (E) N: lymph node stage, and (F) M: metastasis stage between C1 and C2 subtypes are presented.

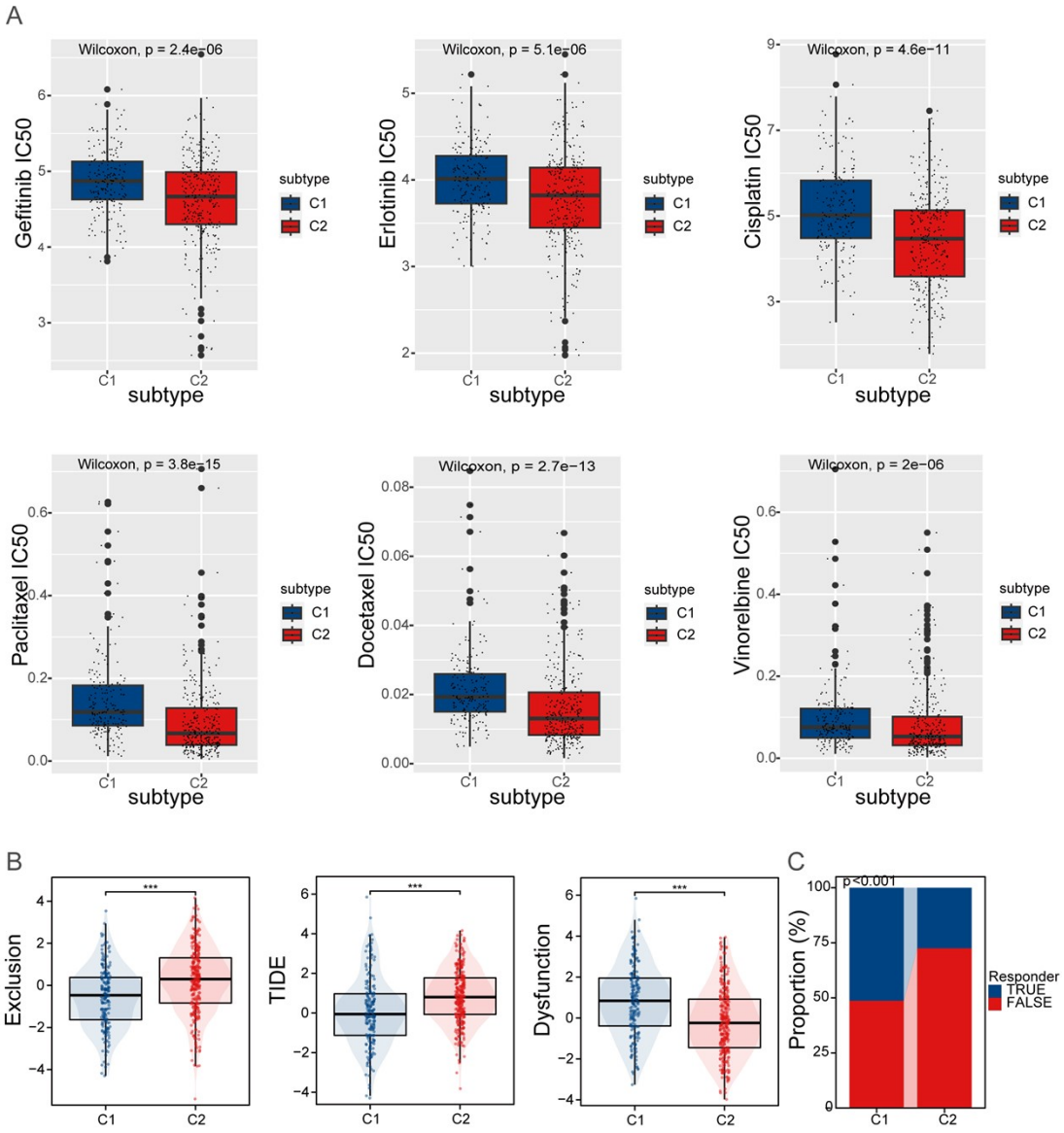

Figure S3. Comparison of efficacy between chemotherapy and immunotherapy in different subtypes. (A) Box plot of IC_{50} estimation for chemotherapy drugs in two subtypes. (B) Exclusion score, TIDE score, and Dysfunction score. (C) Distribution of responders and non-responders to immunotherapy in different subtypes. (***p < 0.001; **p < 0.01; *p < 0.05; ns: not significant).

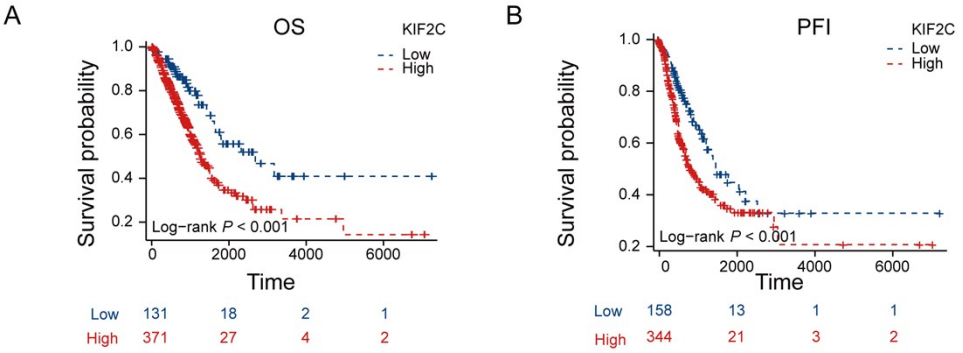

Figure S4. Survival analysis of KIF2C. The surv_cutpoint function from the "survminer" package was utilized to determine the optimal grouping cut-off for survival analysis. Kaplan-Meier (K-M) survival analysis was conducted to assess the differences in (A) overall survival (OS) and (B) progression-free interval (PFI) between the high-risk and low-risk groups.