Supplementary Figures

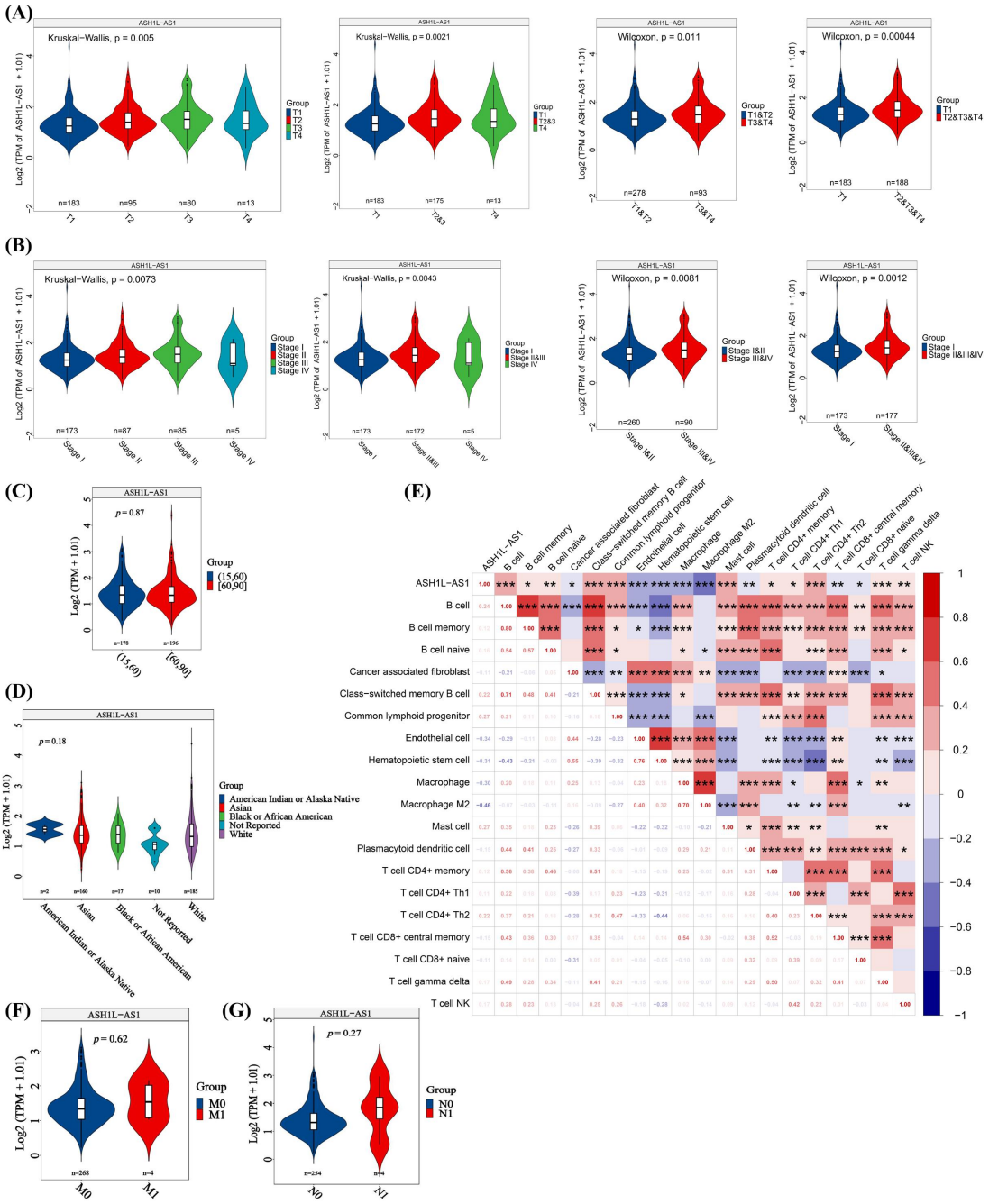

Fig. S1. Associations between *ASH1L-AS1* expression and clinical features, RAS mutation status, and tumor immune microenvironment in HCC. (A – D) Violin plots illustrating the association between *ASH1L-AS1* expression and clinical characteristics in HCC patients, including tumor stage (A), clinical stage (B), age (C), and ethnicity (D). **(E)** Correlation plot (corrplot) showing the relationship between *ASH1L-AS1* expression and the infiltration levels of various stromal and immune cell

types in the tumor microenvironment, as predicted by xCell. **(F – G)** Violin plots showing the association between *ASH1L-AS1* expression and metastasis status (F) and lymph node involvement (G) in HCC patients.

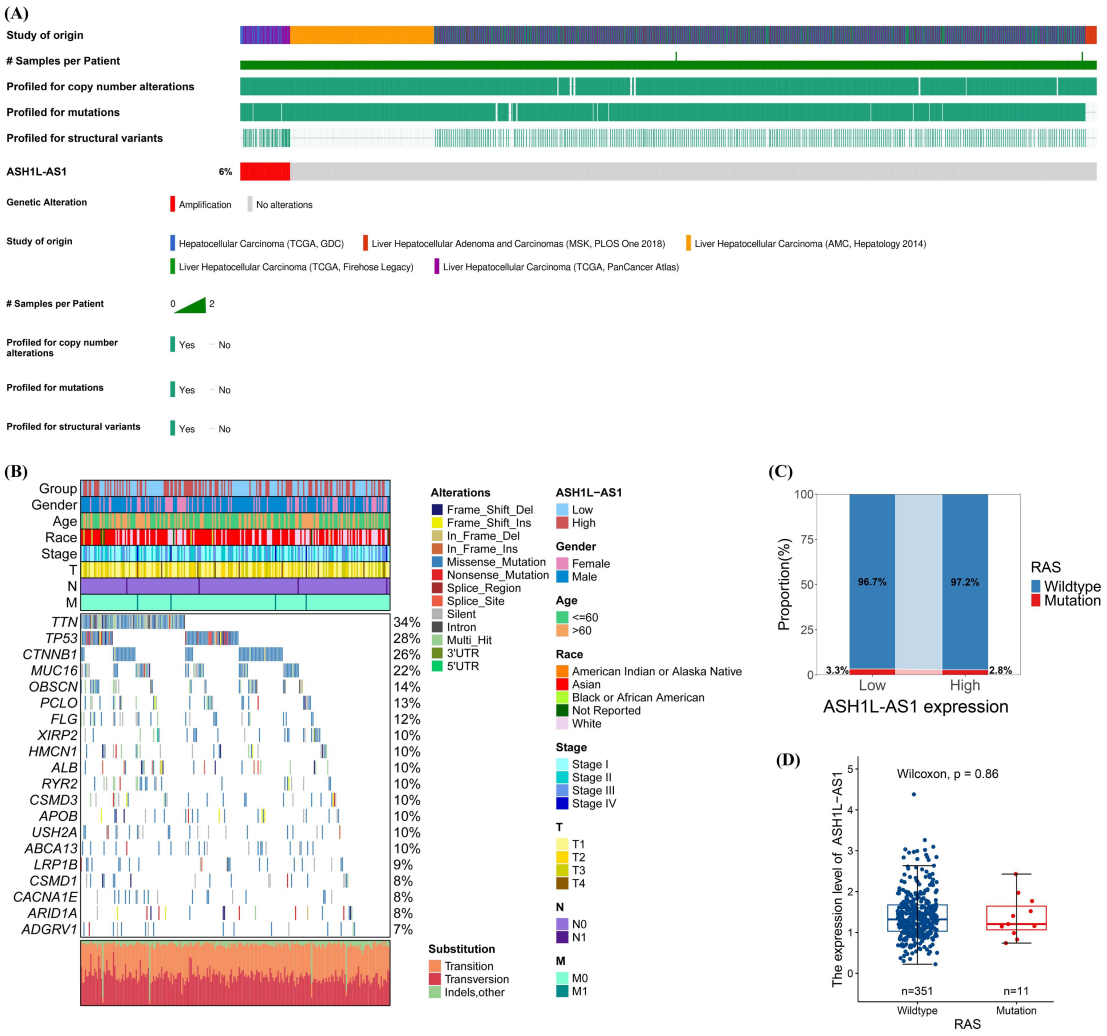

Fig. S2. Genomic alterations and mutation landscape associated with *ASH1L-AS1* in HCC. (A) Analysis of 1,380 HCC patients from five studies in cBioPortal revealed that 6% of patients exhibited *ASH1L-AS1* amplification or structural variations. **(B)** Upregulated *ASH1L-AS1* is associated with higher mutation frequencies in hotspot genes in HCC. **(C)** Stacked bar plot showing the proportion of RAS mutations in HCC patients with high and low *ASH1L-AS1* expression. **(D)** Box plot showing the differential expression of *ASH1L-AS1* between HCC patients with RAS mutations and those with wild-type RAS.

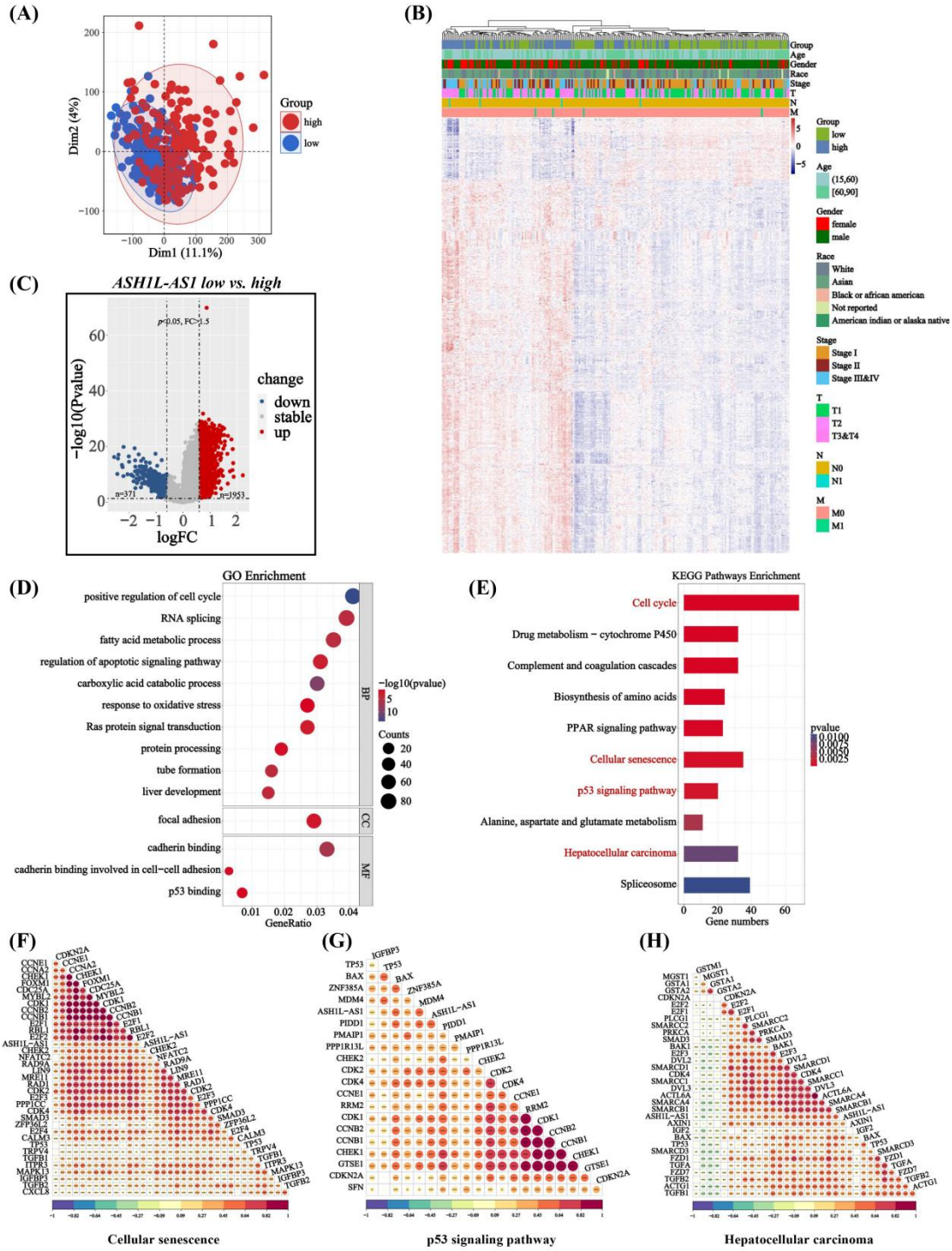

Fig. S3. TCGA-LIHC data analysis reveals distinct gene expression patterns and dysregulated pathways associated with *ASH1L-AS1* expression in HCC. (A-C) PCA plot (A), heatmap (B), and volcano plot (C) showing significant differences in gene expression patterns between HCC patients with high and low *ASH1L-AS1* expression. **(D-E)** GO and KEGG enrichment analyses highlighting the functional pathways enriched in differentially expressed genes between the two groups. **(F-H)**

Correlation plots illustrating the association of enriched genes with cellular senescence (F), p53 signaling (G), and hepatocellular carcinoma-related pathways (H).

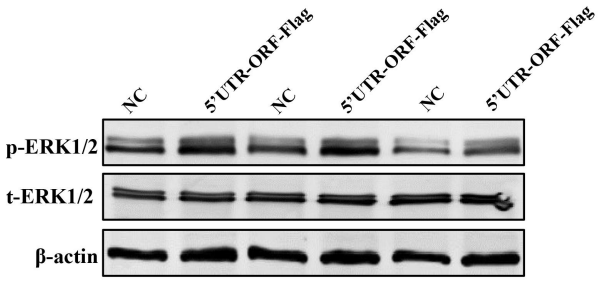

Fig. S4. Overexpression of the microprotein APPLE enhances p-ERK1/2 levels in HepG2 cells.

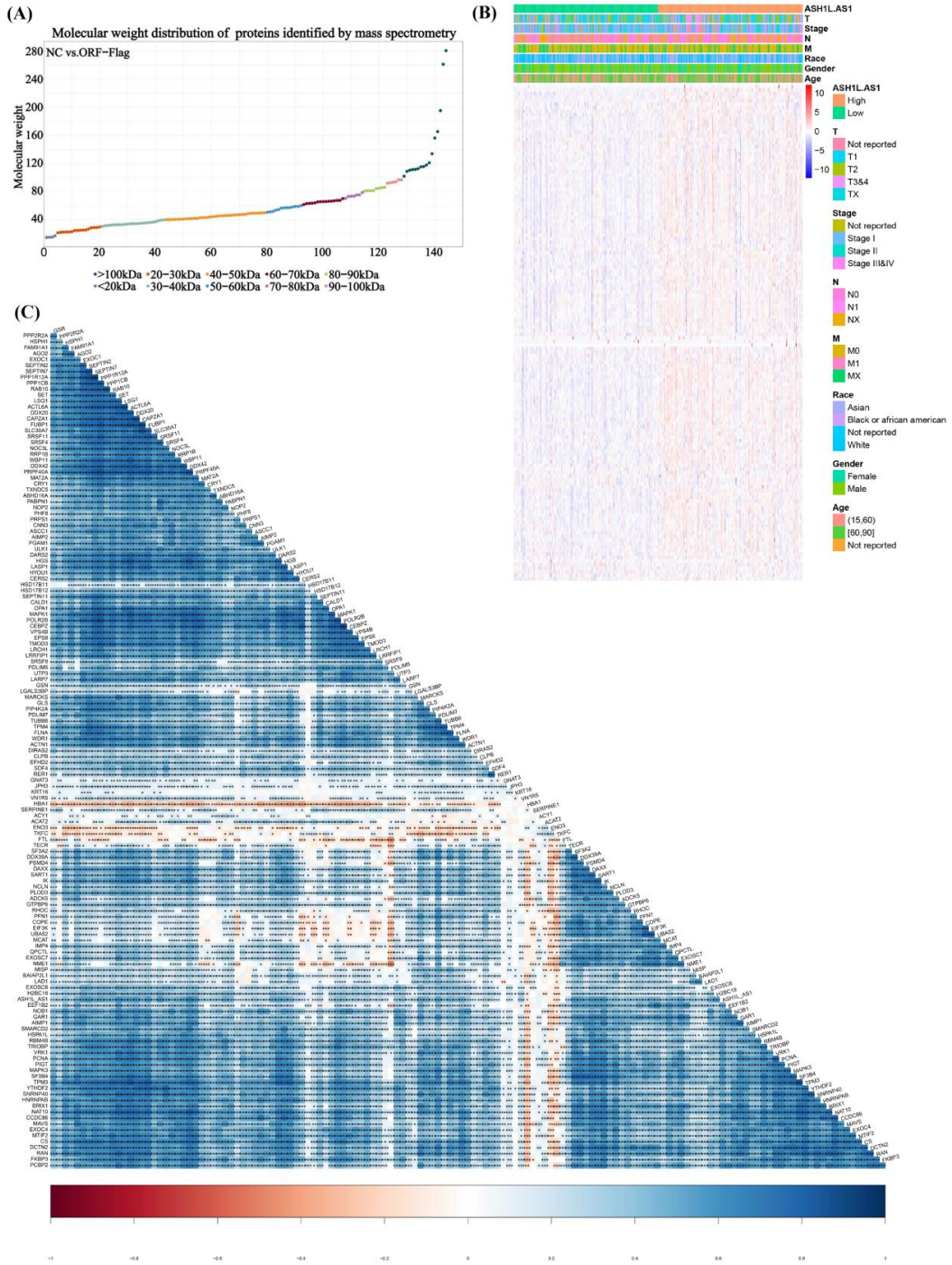

Fig. S5. Expression characteristics of APPLE-Flag-specific interacting proteins and their association with *ASH1L-AS1* in HCC. (A) Molecular weight distribution of proteins specifically interacting with the APPLE-Flag fusion protein. **(B)** TCGA-LIHC analysis reveals that proteins enriched by APPLE-Flag, identified via co-immunoprecipitation and proteomics, are significantly upregulated in HCC tissues with high *ASH1L-AS1* expression. **(C)** Expression levels of these proteins are positively correlated with *ASH1L-AS1* expression across HCC samples.

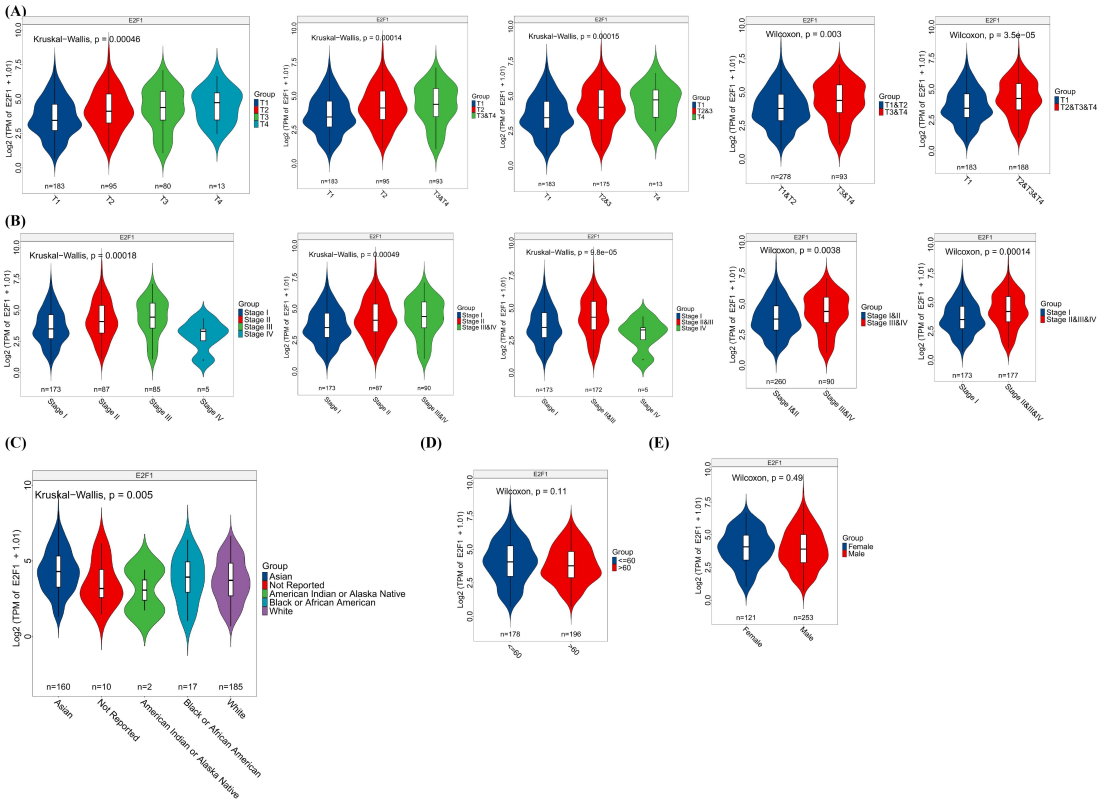

Fig. S6. TCGA-LIHC data analysis reveals the clinical relevance of *E2F1* gene expression in HCC. *E2F1* gene expression is significantly associated with tumor stage **(A)**, clinical stage **(B)**, and ethnicity **(C)**, but shows no significant correlation with age **(D)** or sex **(E)**.