Figure S1. Comprehensive methodological framework for CALCR investigation in liver cancer. The flowchart illustrates the multi-step process employed to examine the role of CALCR in LIHC, spanning bioinformatics and experimental validation. Differential expression and correlation analysis are conducted using TCGA, MSigDB and GTEx datasets, alongside fibrosis gene set enrichment and single-cell analysis from the TISCH2 database. Experimental validation includes cellular assays (transfection, RT-qPCR, apoptosis and migration), with key insights into fibrosis, ferroptosis, and the immune microenvironment derived from genomic and immunotherapy response analyses. CALCR, calcitonin receptor; LIHC, liver hepatocellular carcinoma; RT-qPCR, reverse transcription-quantitative PCR; TCGA, The Cancer Genome Atlas; MSigDB, Molecular Signatures Database; GTEx, Genotype-Tissue Expression; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; ssGSEA, single-sample gene set enrichment analysis; TISCH2, Tumor Immune Single Cell Hub 2.

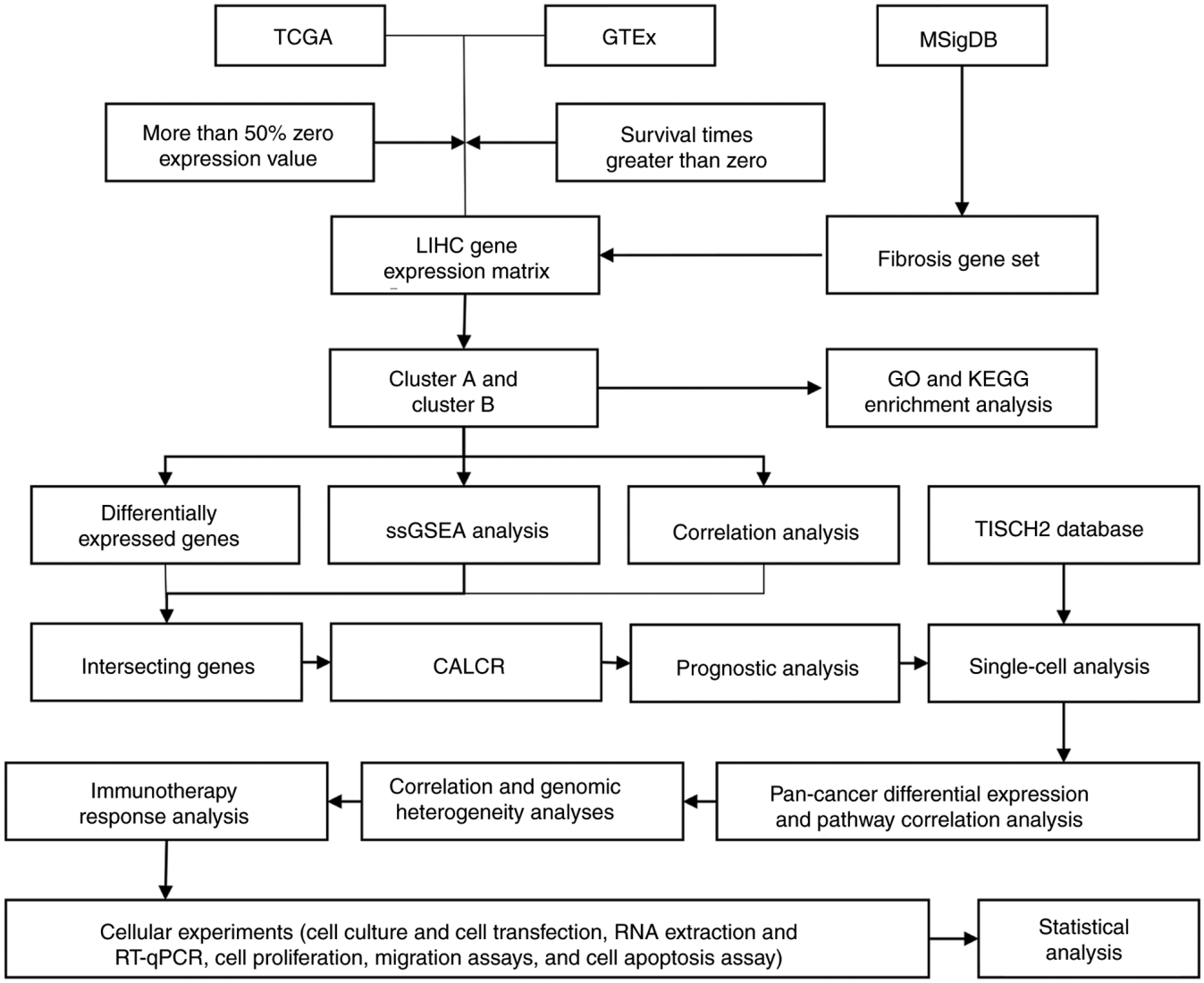

Figure S2. Experimental validation of CALCR expression in normal and cancerous tissues using GEO and HPA databases. The differential expression of CALCR was analyzed to validate bioinformatics findings. GSE142987 dataset analysis showed a significant upregulation of CALCR in tumor tissues compared to normal tissues (P=0.033) (A) Differential expression of CALCR across tumor stages (Stage: 0, Stage: A, Stage: B/C, Stage: n/a) in the GSE142987 dataset. Significant differences in CALCR expression were observed between Stage: n/a and Stage: A, as well as between Stage: A and Stage: B/C. (B) No significant differences were found between Stage: 0 and the other stages. (C and D) Validation using the HPA database indicated no CALCR expression in normal liver tissue (female, age 73), while low CALCR expression was observed in LIHC tissue (male, age 73) with moderate staining in <25% of cells, primarily in the cytoplasmic and membranous regions (magnification, x40). These findings reinforce the differential expression of CALCR in cancerous compared with normal tissues, suggesting its role in tumor progression. *P<0.05, **P<0.01, ***P<0.001. CALCR, calcitonin receptor; GEO, Gene Expression Omnibus; HPA, Human Protein Atlas.