Figure S1. Flow diagram of the experimental design. Relevant experiments were performed *in vivo* and *in vitro* after knockdown of TMED3 in order to investigate the effect of TMED3 on chordoma cells. Reverse transcription-qPCR and western blot assays were performed to determine the expression levels. Differentially expressed proteins were detected using an apoptosis antibody array. TMED3, transmembrane Emp24 protein transport domain containing 3; qPCR, quantitative PCR.

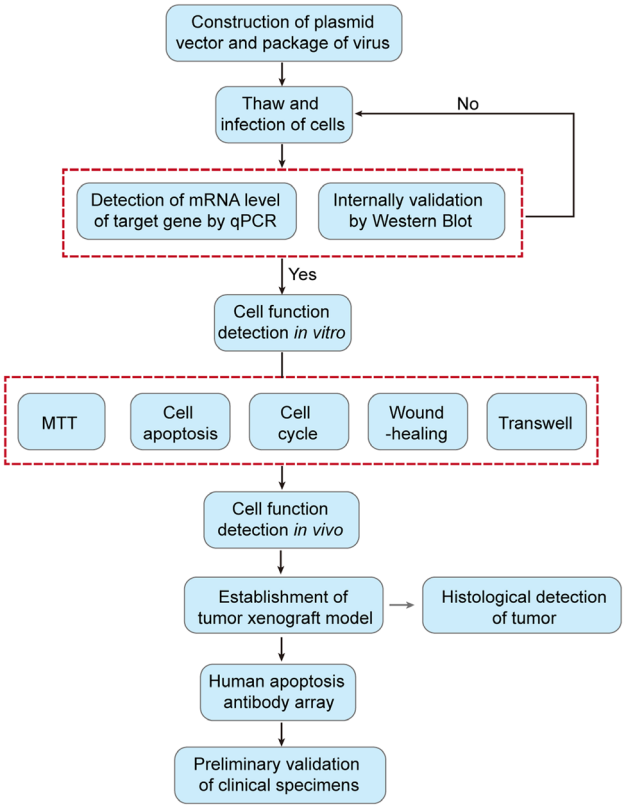

Figure S2. Preliminary validation of TMED3 expression in clinical chordoma specimens. Immunohistochemical staining reveals moderate staining for TMED3 mainly located in the cytoplasm in chordoma tissue. Scale bar, 50 μm. TMED3, transmembrane Emp24 protein transport domain containing 3.

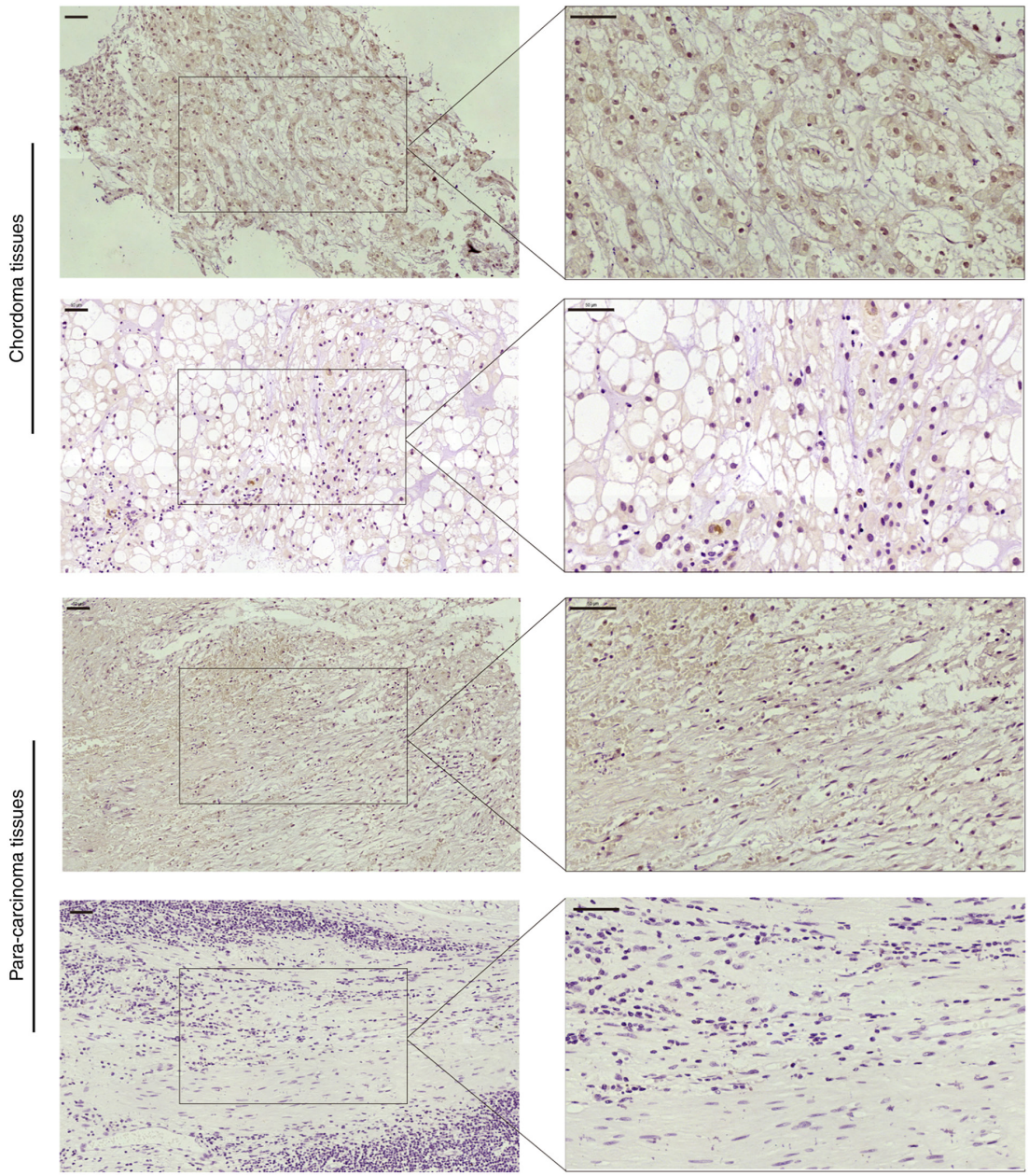

Figure S3. Proposed effects of TMED3 on the proliferation and survival of chordoma cells. Knockdown of TMED3 inhibited cell proliferation and migration, and promoted apoptosis; the differentially expressed genes are proposed to be involved. TMED3, transmembrane Emp24 protein transport domain containing 3; shRNA, short hairpin RNA; HSP27, heat shock protein 27; IGF, insulin-like growth factor, IGFBP2, IGF binding protein 2; p, phosphorylated.