Supplemental Materials and Methods

Gene set enrichment analysis (GSEA)

In this study, CRC expression profiling files downloaded from GEO dataset GSE38832 was analyzed by GSEA. GSEA was performed using the GSEA v2.0.13 software. Gene sets M7561 was used for enrichment of mTOR signaling pathway-related genes. All gene set files for this analysis were obtained from GSEA website (www.broadinstitute.org/gsea/). Enrichment map was used for visualization of the GSEA results. Enrichment score (ES) and False discovery rate (FDR) value were applied to sort mTOR pathway enriched after gene set permutations were performed 1000 times for the analysis.

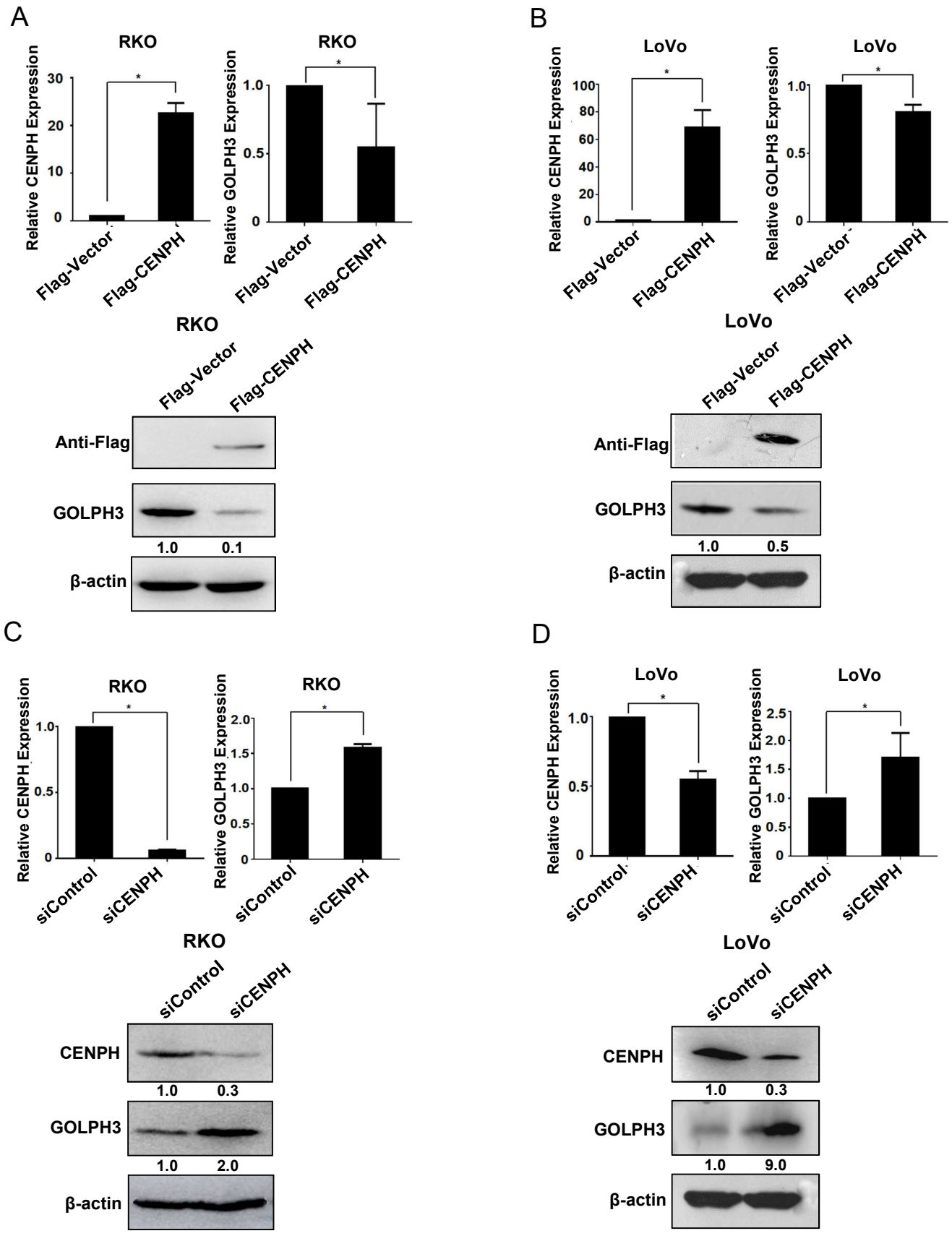

Fig. S1. Real-time PCR and Western blot confirmed the efficiency of CENPH overexpression and knockdown in CRC cells.

(A, B) Overexpression of CENPH by transfection of pCMV-3Tag-1A-CENPH plasmid increased the mRNA and protein levels of CENPH, but decreased the levels of GOLPH3 in RKO and LoVo cells. (C, D) Knockdown of CENPH by siRNAs reduced CENPH mRNA and protein levels, but upregulated GOLPH3 expression in CRC cells. For Western blot, ratios of CENPH and GOLPH3/β-actin shown under the representative blots were normalized to that of controls in each cell line. Upper panel, mRNA level; lower panel, protein level.

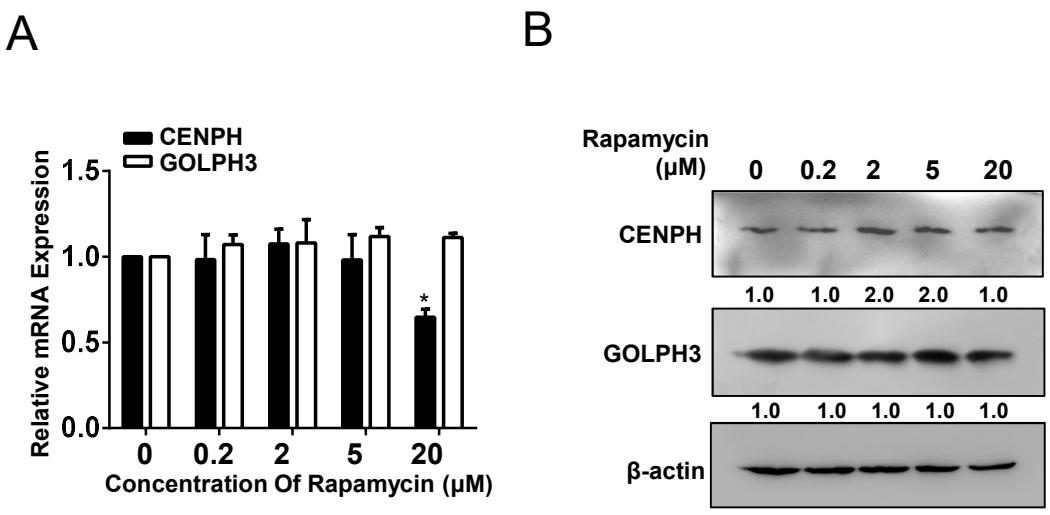

Fig. S2. Expression of CENPH and GOLPH3 in LoVo cells treated with rapamycin.

CENPH and GOLPH3 expression were determined by Real-time PCR (A) and Western blot (B), respectively, in LoVo treated with increasing concentration of rapamycin. *, $P < 0.05$.

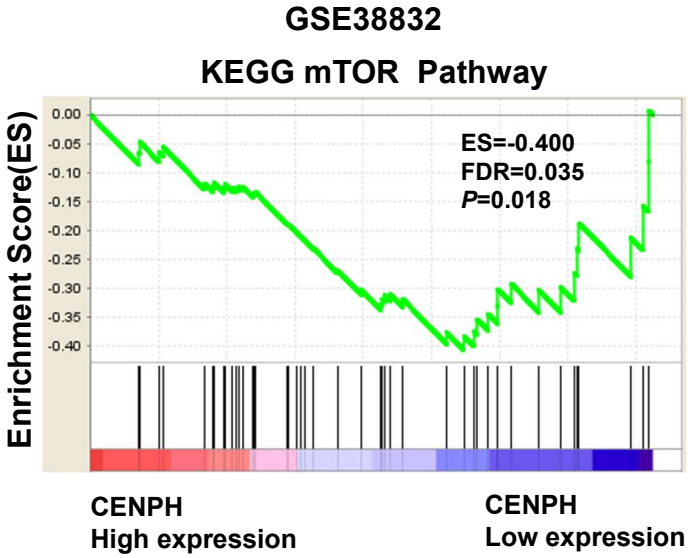

Fig. S3. GSEA analysis of mTOR signaling pathway based on the gene expression profiles of high CENPH group (red) versus low CENPH group (blue) in GSE38832.

ES, enrichment score; FDR, false discovery rate value.