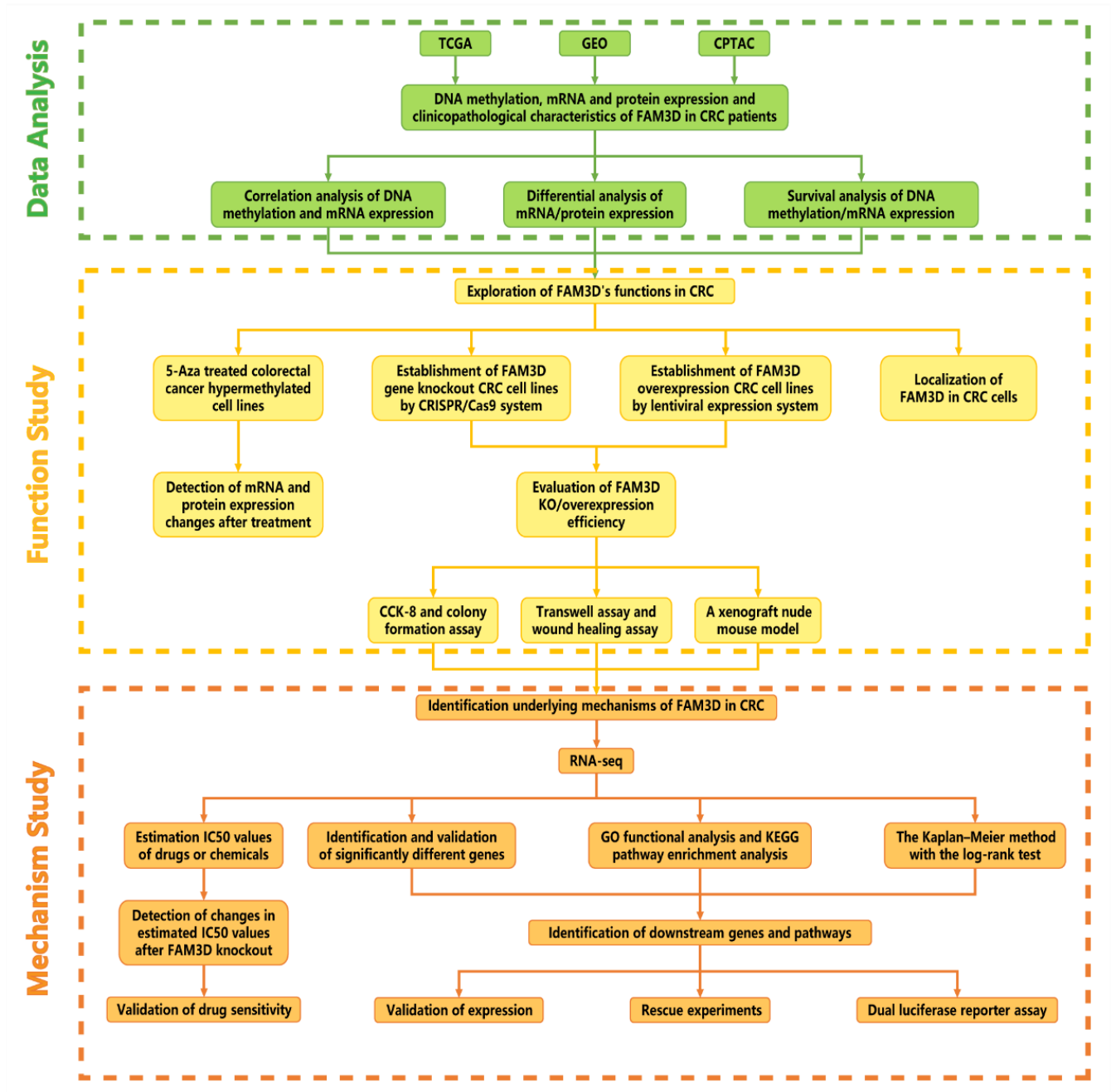
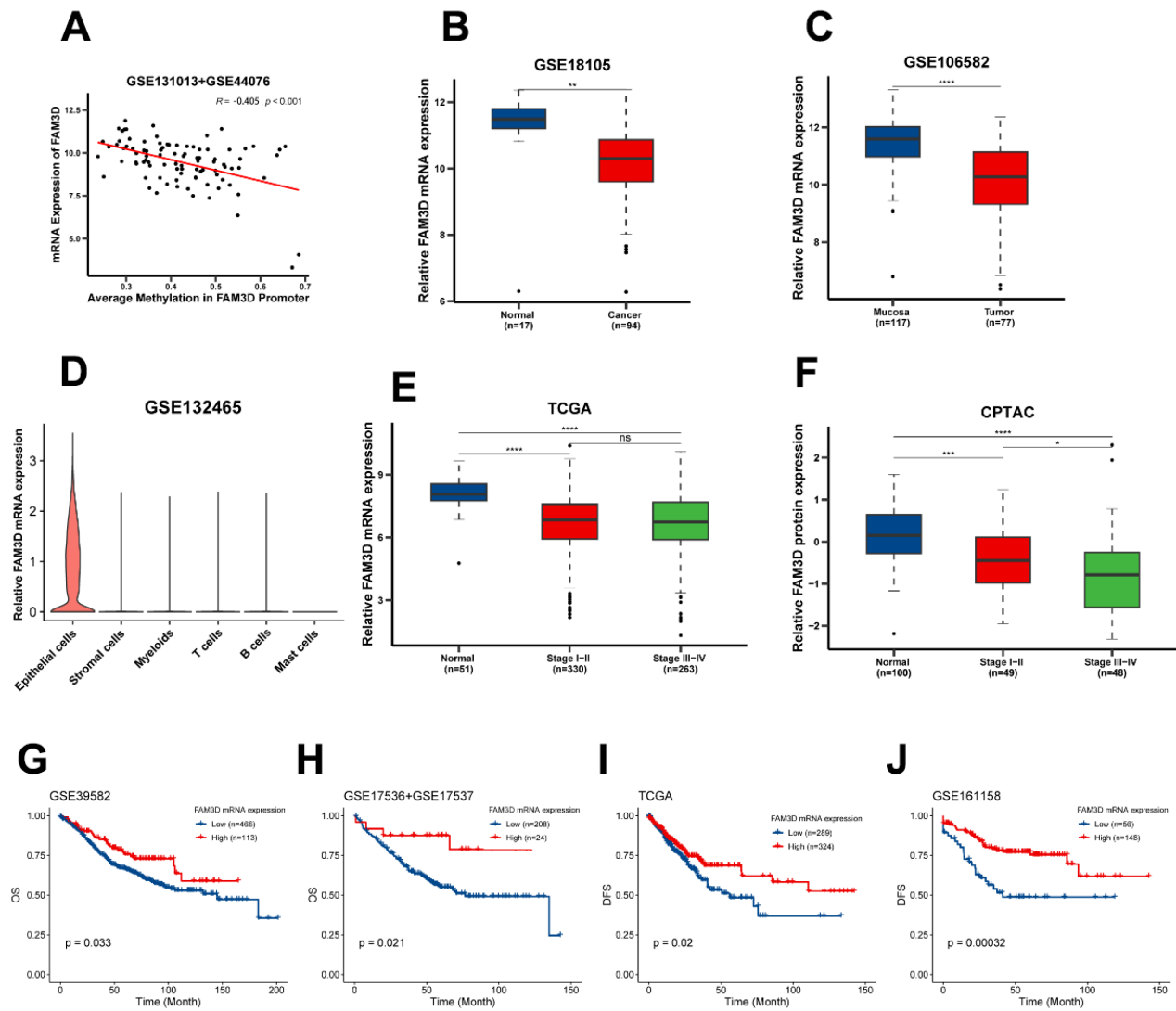


SUPPLEMENTARY FIGURES



Supplementary Figure 1. A workflow of the present study.

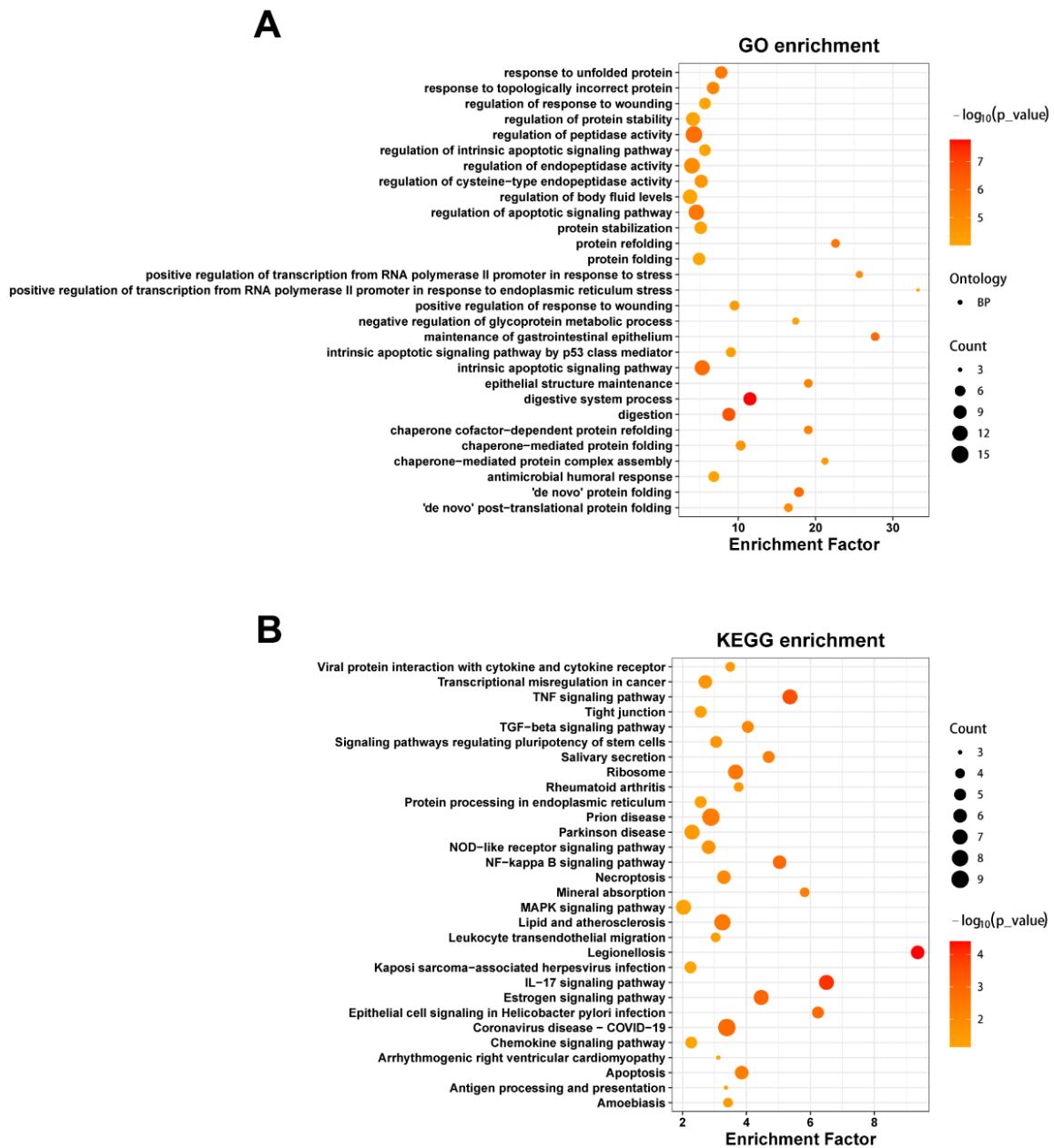


Supplementary Figure 2. Methylation, expression and prognosis of *FAM3D*. (A) Correlation between methylation of *FAM3D* promoter and its mRNA expression in GEO cohorts (GSE131013 + GSE44076). (B, C) The mRNA expression of *FAM3D* in adjacent normal tissues and CRC tissues in (B) GSE18105 and (C) GSE106582. (D) *FAM3D* mRNA expression in different cell types in GSE132465. (E, F) *FAM3D* mRNA and protein expression in different tumor stages in TCGA (E, mRNA expression) and CPTAC (F, protein expression). (G, H) Kaplan-Meier curve of OS based on the *FAM3D* expression in tumor tissues of GEO cohorts (G: GSE39582; H: GSE17536 + GSE17537). (I, J) Kaplan-Meier curve of DFS based on the *FAM3D* expression in tumor tissues in (I) TCGA and (J) GSE161158. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

WT (131177)	21143	CCCCA ^{sgRNA1} GAGGTTAAAAAGTACAAGT ^{PAM} GTGGCCTCATCAAGCCCTGCCAGCCAACACTACTTTG	21202
LoVo	WT	CCCCA ^{sgRNA1} GAGGTTAAAAAGTACAAGT ^{PAM} GTGGCCTCATCAAGCCCTGCCAGCCAACACTACTTTG	
	NC	CCCCA ^{sgRNA1} GAGGTTAAAAAGTACAAGT ^{PAM} GTGGCCTCATCAAGCCCTGCCAGCCAACACTACTTTG	
	KO1	C-----ATCAAGCCCTGCCAGCCAACACTACTTTG	
HT29	WT	CCCCA ^{sgRNA1} GAGGTTAAAAAGTACAAGT ^{PAM} GTGGCCTCATCAAGCCCTGCCAGCCAACACTACTTTG	
	NC	CCCCA ^{sgRNA1} GAGGTTAAAAAGTACAAGT ^{PAM} GTGGCCTCATCAAGCCCTGCCAGCCAACACTACTTTG	
	KO1	CCCCA ^{sgRNA1} GAGGTTAAAAAGTACAAGT ^{PAM} GTGGCCTCATCAAGCCCTGCCAGCCAACACTACTTTG	
WT (131177)	21229	AACGTCGTGGGCCCTACTATGTGCTTTGAAGA ^{PAM} CCGCATGTAAGTACCTAAGACACA ^{sgRNA2} AACTA	21288
LoVo	WT	AACGTCGTGGGCCCTACTATGTGCTTTGAAGACCGCATGTAAGTACCTAAGACACA	
	NC	AACGTCGTGGGCCCTACTATGTGCTTTGAAGACCGCATGTAAGTACCTAAGACACA	
	KO2	AACGTCGTGGGCCCTACTATGTGCTTTGAAG -----TACCTAAGACACA	
HT29	WT	AACGTCGTGGGCCCTACTATGTGCTTTGAAGACCGCATGTAAGTACCTAAGACACA	
	NC	AACGTCGTGGGCCCTACTATGTGCTTTGAAGACCGCATGTAAGTACCTAAGACACA	
	KO2	AACGTCGTGGGCCCTACTATGTGCTTTGAAGACC-----AACTA	

Red: sgRNA; Blue: PAM; Green: Insertion

Supplementary Figure 3. Genomic DNAs from four cell types (WT *FAM3D*, NC, KO1, and KO2 cells) of LoVo and HT29 cells were isolated and PCR amplicons flanking the CRISPR/Cas9-targeted regions were sequenced. Sequence alignments of bases 21143–21202 and 21229–21288 of the *FAM3D* gene are shown. Numbering is based on the entire *FAM3D* gene sequence (NCBI Gene: 131177).



Supplementary Figure 4. Bioinformatics analysis for *FAM3D* overexpression CRC cells in GSE132465. (A) GO functional analysis showed the top 29 biological processes that are enriched in DEGs. (B) KEGG enrichment analysis showed the top 30 signaling pathways that are enriched in DEGs.