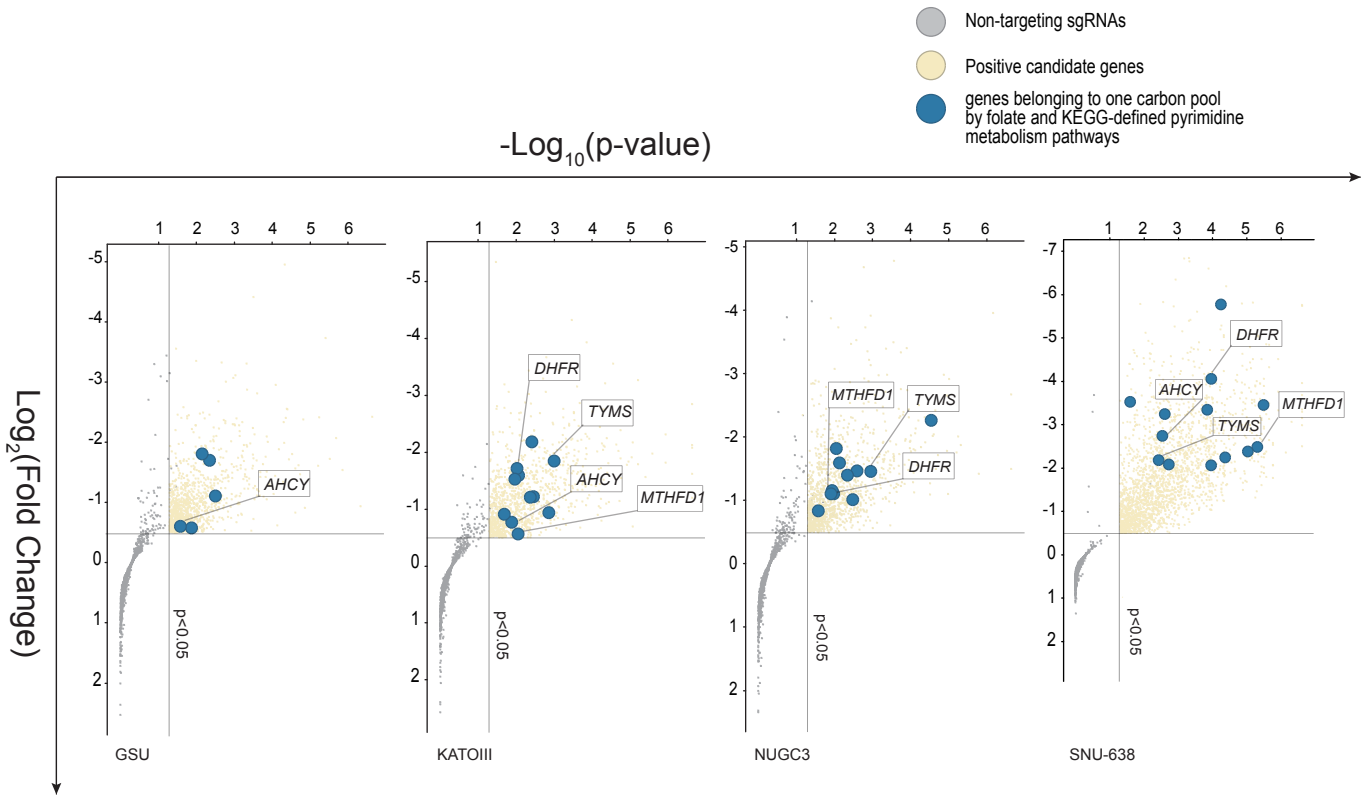
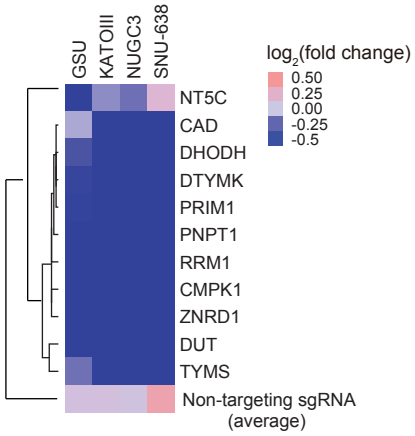
MYC predetermines the sensitivity of gastrointestinal cancer to antifolate drugs through regulating *TYMS* transcription

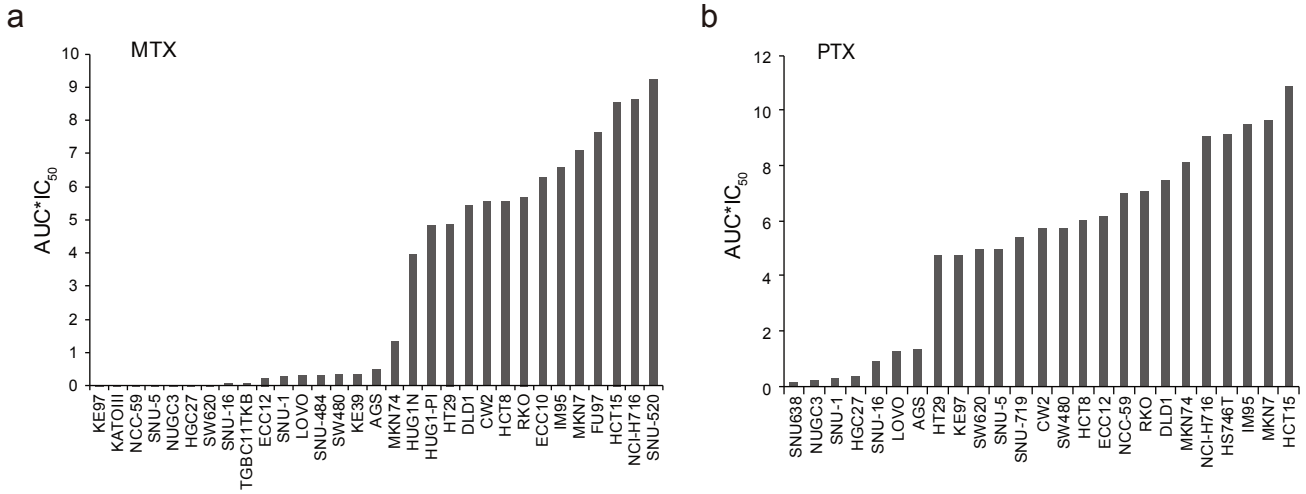
1. Supplementary Figures 1-7



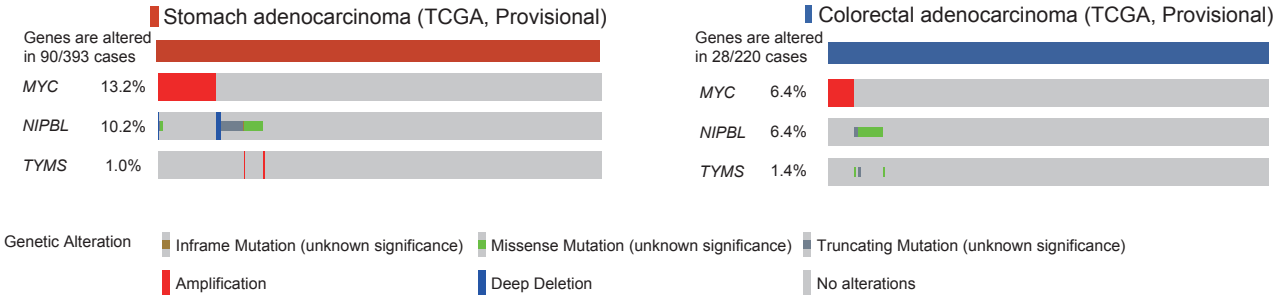
Supplementary Fig. 1. Volcano plot of selected genes and nontargeting sgRNAs in $DMSO_{Day14}$ vs $DMSO_{Day0}$ of four gastric cancer cell lines. Grey dots indicated non-targeting sgRNAs, and yellow dots indicated genes with $\log_2$(fold change)<-0.5 and p<0.05. Genes involved in one carbon pool by folate and KEGG-defined pyrimidine metabolism pathways were shown in blue dots. 1,000 non-targeting sgRNAs were used as control in all the four independent screening.



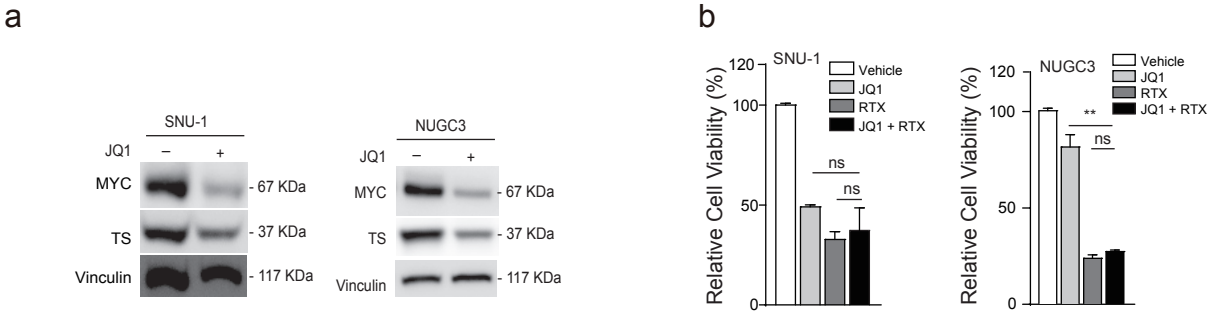
Supplementary Fig. 2. sgRNAs targeting genes in KEGG-defined pyrimidine metabolism pathway were significantly decreased after 14 days of cell culture. The average of 1,000 non-targeting sgRNAs was used as control.



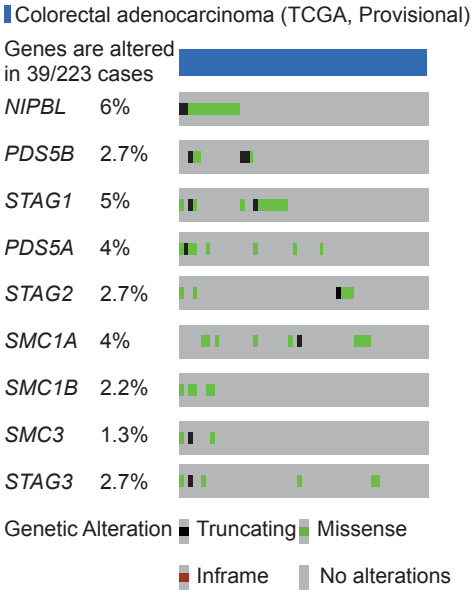
Supplementary Fig. 3. The cell viability of multiple gastrointestinal cancer cell lines under the treatment of MTX (a) and PTX (b). IC_{50} multipled by AUC of MTX and PTX treatment was presented. The IC_{50} and AUC were calculated according to dose response curves, respectively.



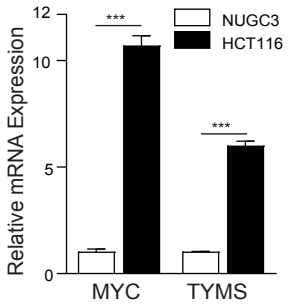
Supplementary Fig. 4. *MYC* and *NIPBL* are discretely mutated in both stomach and colorectal adenocarcinomas of TCGA provisional database. There're 53 *MYC*-amplified, 48 *NIPBL*-mutated and 4 *TYMS*-amplified cases in 393 stomach adenocarcinomas, and 14 *MYC*-amplified, 14 *NIPBL*-mutated and 3 *TYMS*-mutated cases in 220 colorectal adenocarcinomas. Tumour samples of TCGA provisional database with both mutation and copy number alternations were used in this study, without accounting *NIPBL*-amplified cases.



Supplementary Fig. 5. JQ1 significantly reduced the cell viability of SNU-1 and NUGC3 cell lines. (a) Immunoblotting analysis of the protein expression levels of MYC and thymidylate synthase in SNU-1 and NUGC3 after 1 μM JQ1 treatment for 72 hours. (b) The relative cell viability of SNU-1 and NUGC3 after the treatment of indicated drugs. 10 μM RTX and 10 μM JQ1 were used to achieve the maximal inhibition.



Supplementary Fig. 6. Mutation status of cohesin complex and -associated members in colorectal adenocarcinomas from TCGA provisional database. 223 tumour samples are available for analysing the mutation status of cohesin complex members.



Supplementary Fig. 7. The relative MYC and TYMS mRNA expression levels of HCT116 cells to NUGC3 cells. The mRNA expression levels were detected by q-PCR, normalized by GAPDH.