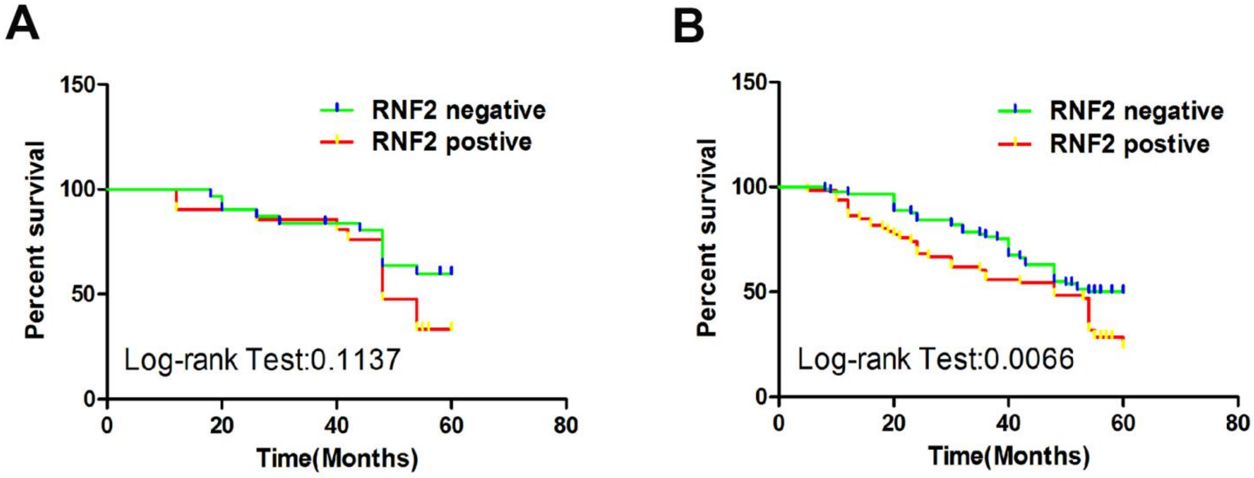
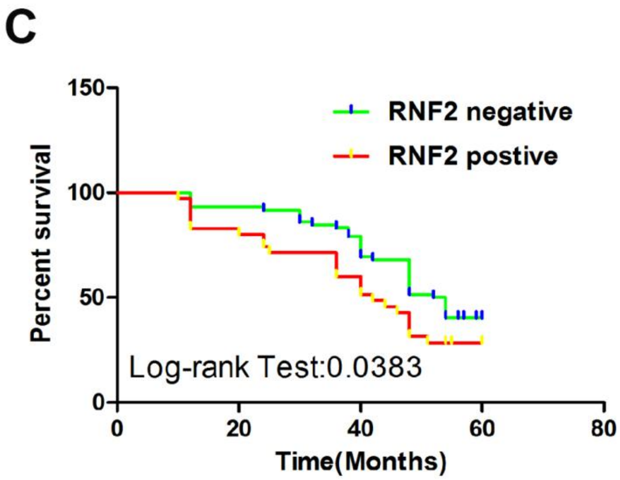
## SUPPLEMENTARY FIGURES

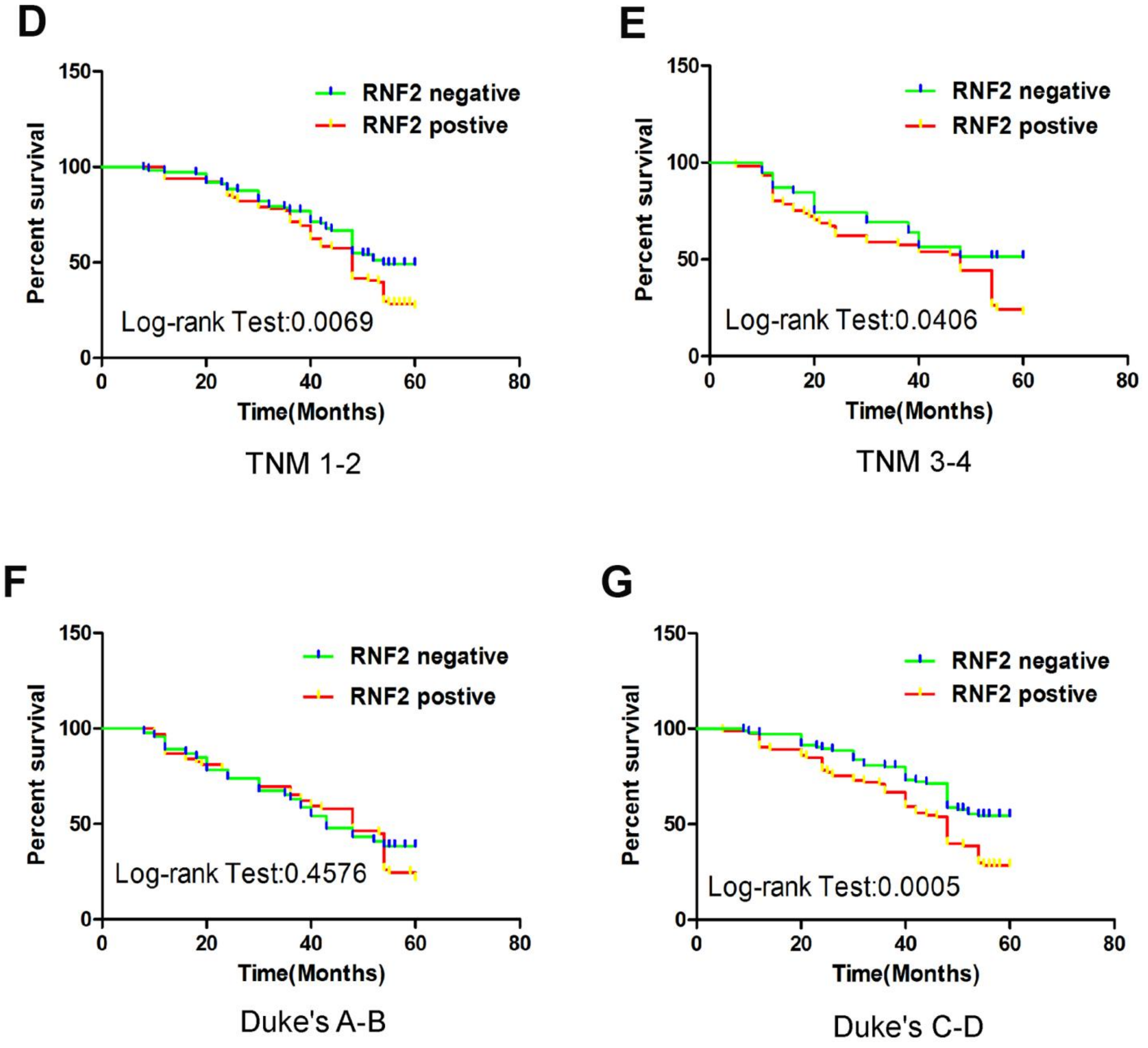


Well differentiated

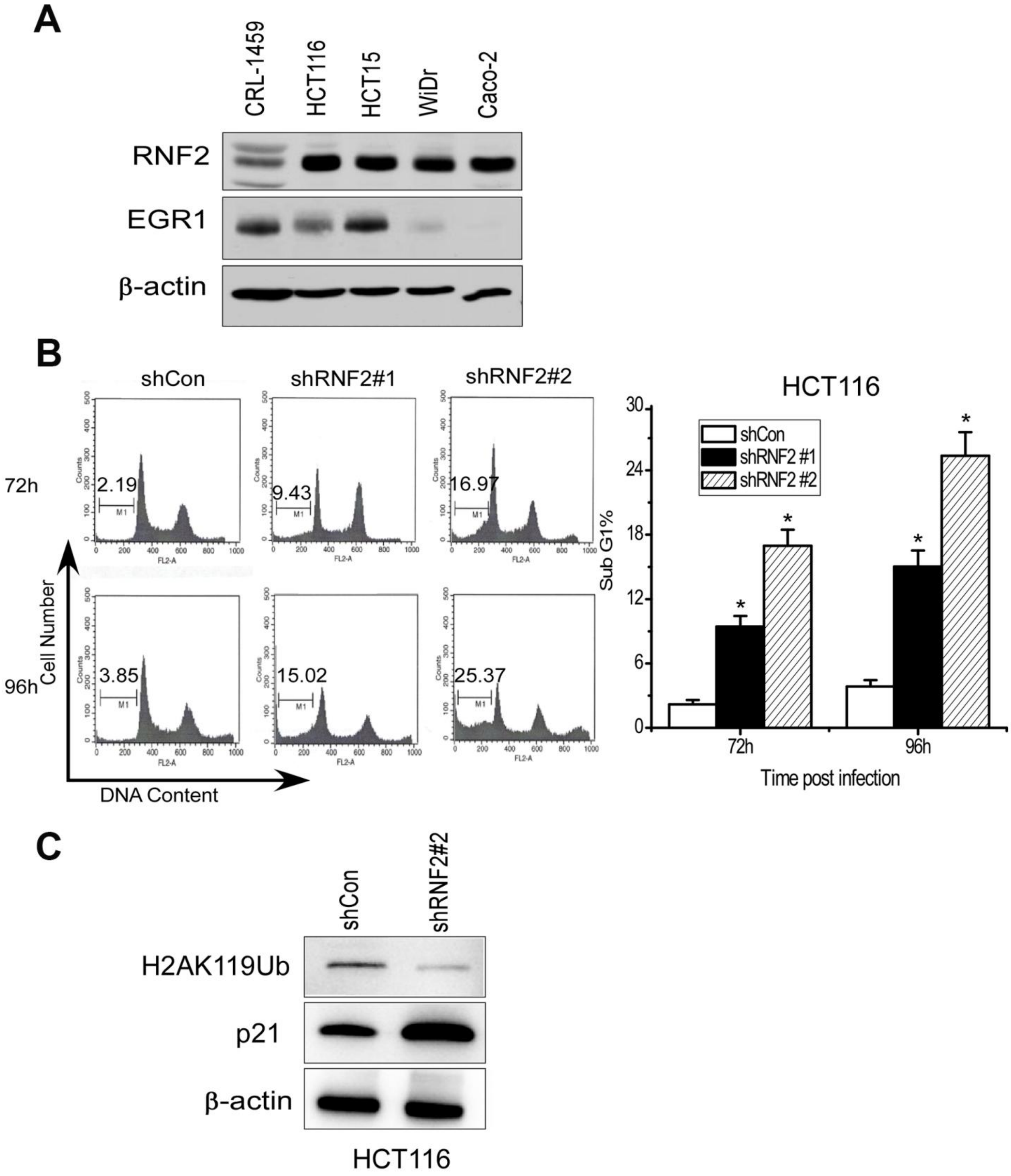
Moderately differentiated



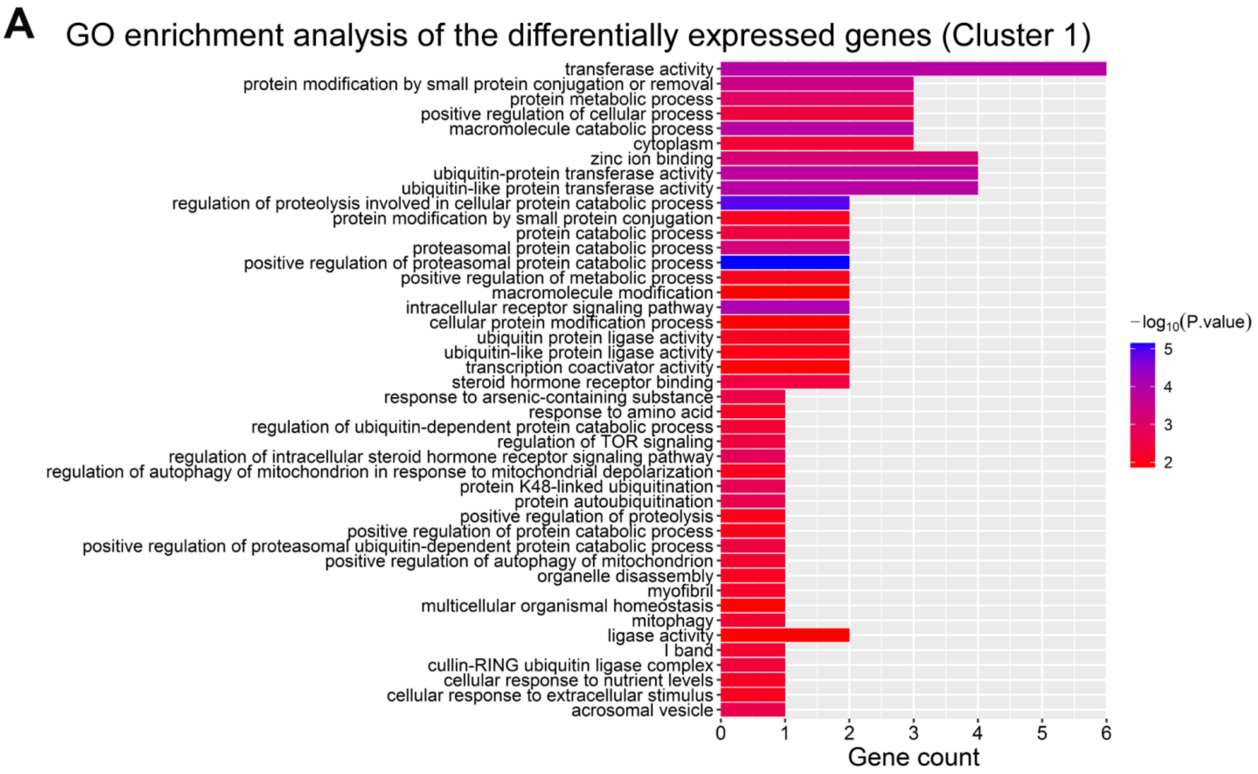
Poorly differentiated

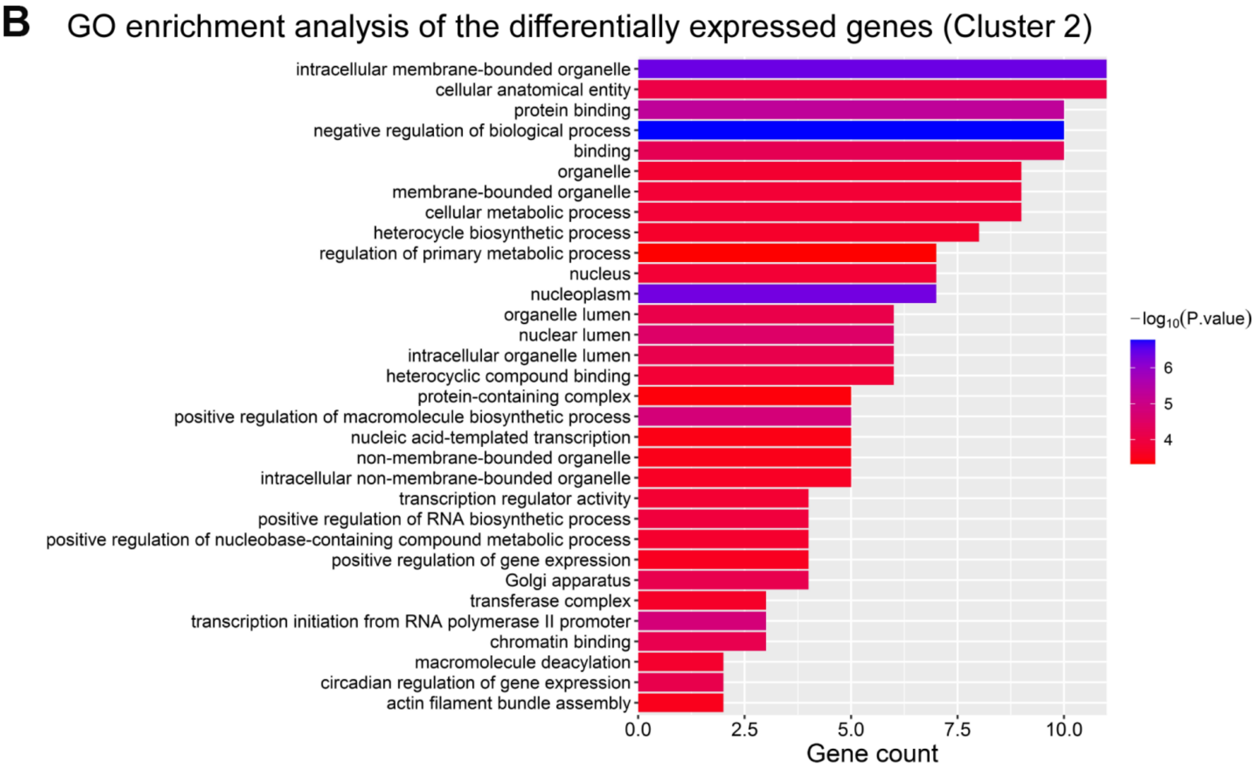


**Supplementary Figure 1. The correlation between RNF2 expression and survival in patients with different differentiation status, TNM stage or Duke's stage.** Patients' survival was calculated and plotted using Kaplan-Meier method. Patients were determined as RNF2 positive or RNF2 negative based on RNF2 IHC staining result. (**A**–**C**) Survival of patients with different differentiation status. In patients with moderately differentiated and poorly differentiated tumors, those had positive RNF2 expression showed worse survival. (**D**, **E**) Survival of patients in different TNM stage. In patients with tumors either in TNM 1-2 or TNM 3-4 stage, those had positive RNF2 expression showed worse survival. (**F**, **G**) Survival of patients in different Duke's stage. In patients with tumors in Duke's (**C**, **D**) stage, those had positive RNF2 expression showed worse survival.



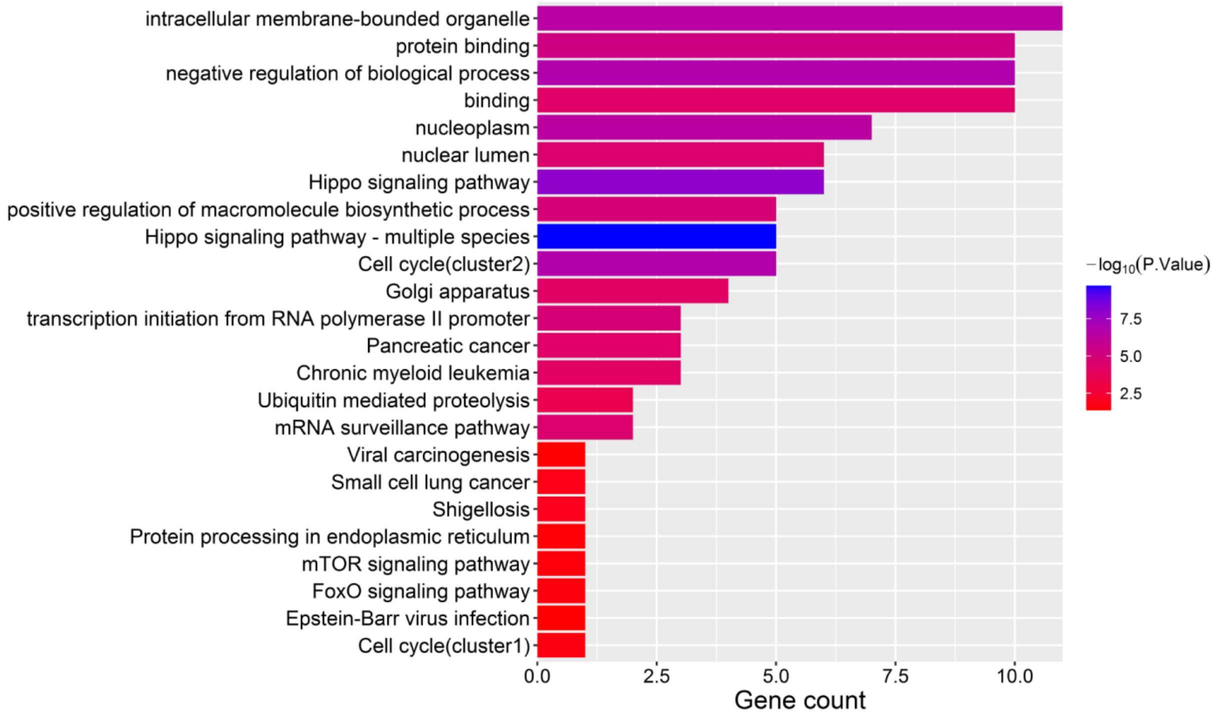
**Supplementary Figure 2. Expression of RNF2 and EGR1 in CRC cells, and knockdown of RNF2 induced apoptosis in HCT116 cells.** (**A**) Western blot analysis to show the expression of RNF2 and EGR1 in several CRC cell lines and normal colon cell CRL-1459. (**B**) Cell cycle analysis of control and RNF2 knockdown HCT116 cells. Data are shown as the mean±S.D. from three independent experiments. *$p<0.05$ versus shCon. (**C**) Western blot analysis to show the expression of p21 and H2AK119Ub in control and RNF2 knockdown HCT116 cells.


A
GO enrichment analysis of the differentially expressed genes (Cluster 1)
transferase activity
protein modification by small protein conjugation or removal
protein metabolic process
positive regulation of cellular process
macromolecule catabolic process
cytoplasm
zinc ion binding
ubiquitin-protein transferase activity
ubiquitin-like protein transferase activity
regulation of proteolysis involved in cellular protein catabolic process
protein modification by small protein conjugation
protein catabolic process
proteasomal protein catabolic process
positive regulation of proteasomal protein catabolic process
positive regulation of metabolic process
macromolecule modification
intracellular receptor signaling pathway
cellular protein modification process
ubiquitin protein ligase activity
ubiquitin-like protein ligase activity
transcription coactivator activity
steroid hormone receptor binding
response to arsenic-containing substance
response to amino acid
regulation of ubiquitin-dependent protein catabolic process
regulation of TOR signaling
regulation of intracellular steroid hormone receptor signaling pathway
regulation of autophagy of mitochondrion in response to mitochondrial depolarization
protein K48-linked ubiquitination
protein autoubiquitination
positive regulation of proteolysis
positive regulation of protein catabolic process
positive regulation of proteasomal ubiquitin-dependent protein catabolic process
positive regulation of autophagy of mitochondrion
organelle disassembly
myofibril
multicellular organismal homeostasis
mitophagy
ligase activity
I band
cullin-RING ubiquitin ligase complex
cellular response to nutrient levels
cellular response to extracellular stimulus
acrosomal vesicle
0
1
2
3
4
5
6
Gene count
$-\log_{10}$(P.value)
5
4
3
2

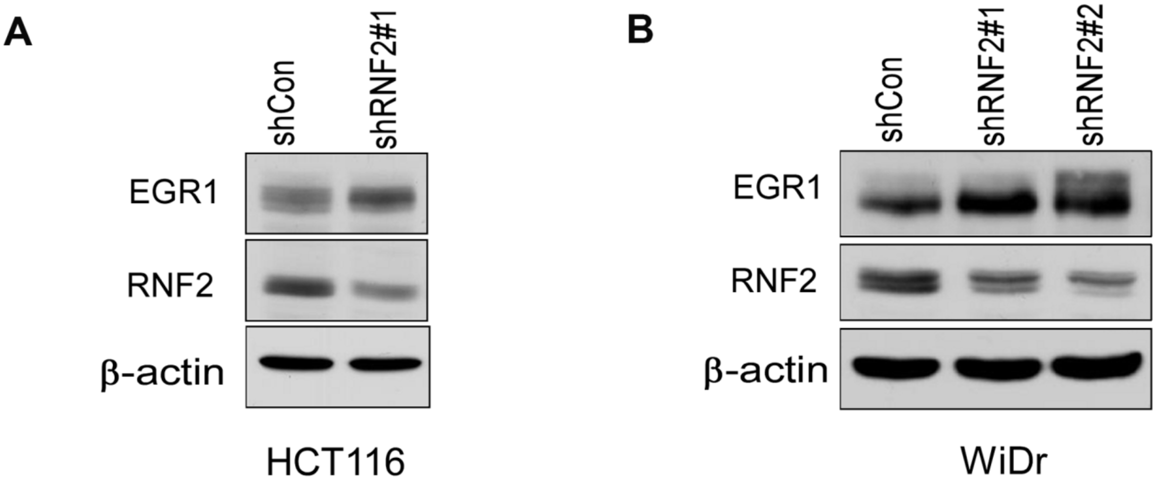

B
GO enrichment analysis of the differentially expressed genes (Cluster 2)
intracellular membrane-bounded organelle
cellular anatomical entity
protein binding
negative regulation of biological process
binding
organelle
membrane-bounded organelle
cellular metabolic process
heterocycle biosynthetic process
regulation of primary metabolic process
nucleus
nucleoplasm
organelle lumen
nuclear lumen
intracellular organelle lumen
heterocyclic compound binding
protein-containing complex
positive regulation of macromolecule biosynthetic process
nucleic acid-templated transcription
non-membrane-bounded organelle
intracellular non-membrane-bounded organelle
transcription regulator activity
positive regulation of RNA biosynthetic process
positive regulation of nucleobase-containing compound metabolic process
positive regulation of gene expression
Golgi apparatus
transferase complex
transcription initiation from RNA polymerase II promoter
chromatin binding
macromolecule deacylation
circadian regulation of gene expression
actin filament bundle assembly
0.0
2.5
5.0
7.5
10.0
Gene count
$-\log_{10}$(P.value)
6
5
4

**C** GO enrichment analysis of the differentially expressed genes (Cluster 3)

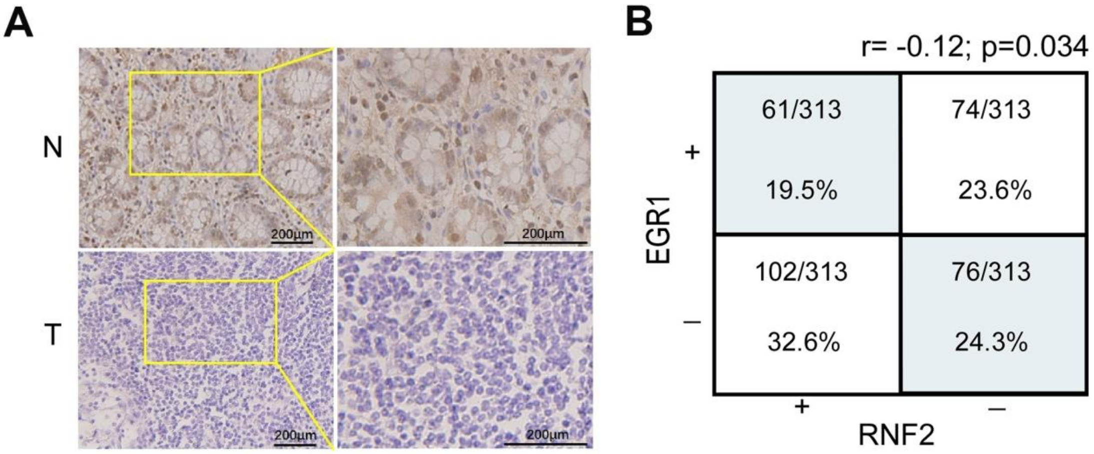
**D** KEGG pathway enrichment analysis of the differentially expressed genes



**Supplementary Figure 3. GO biological process and KEGG pathway enrichment for the differentially expressed genes.** (**A**–**C**) GO enrichment analysis of the differentially expressed genes in RNF2 knockdown cells (Cluster 1-3). The x axis indicates the number of genes within each GO term. (**D**) KEGG pathway enrichment analysis of the differentially expressed genes in RNF2 knockdown cells. The x axis indicates the number of genes within each KEGG term.



**Supplementary Figure 4. Downregulation of RNF2 resulted in upregulation of EGR1 in both HCT116 and WiDr cells.** (**A**) Western blot to show the increased EGR1 expression in RNF2 knockdown HCT116 cells (by shRNF2 #1). (**B**) Western blot to show the increased EGR1 expression in RNF2 knockdown WiDr cells.



**Supplementary Figure 5. Correlation analysis of RNF2 and EGR1 expression in clinical CRC tissues.** (**A**) Representative IHC staining results to show negative and positive EGR1 expression in clinical CRC tumor tissues and adjacent normal tissues. Right pictures are the magnified view of the yellow box in the left. Scale bar: 200 µm. (**B**) Correlation analysis of RNF2 and EGR1 expression in clinical CRC tissues with r = -0.12 and p= 0.034.