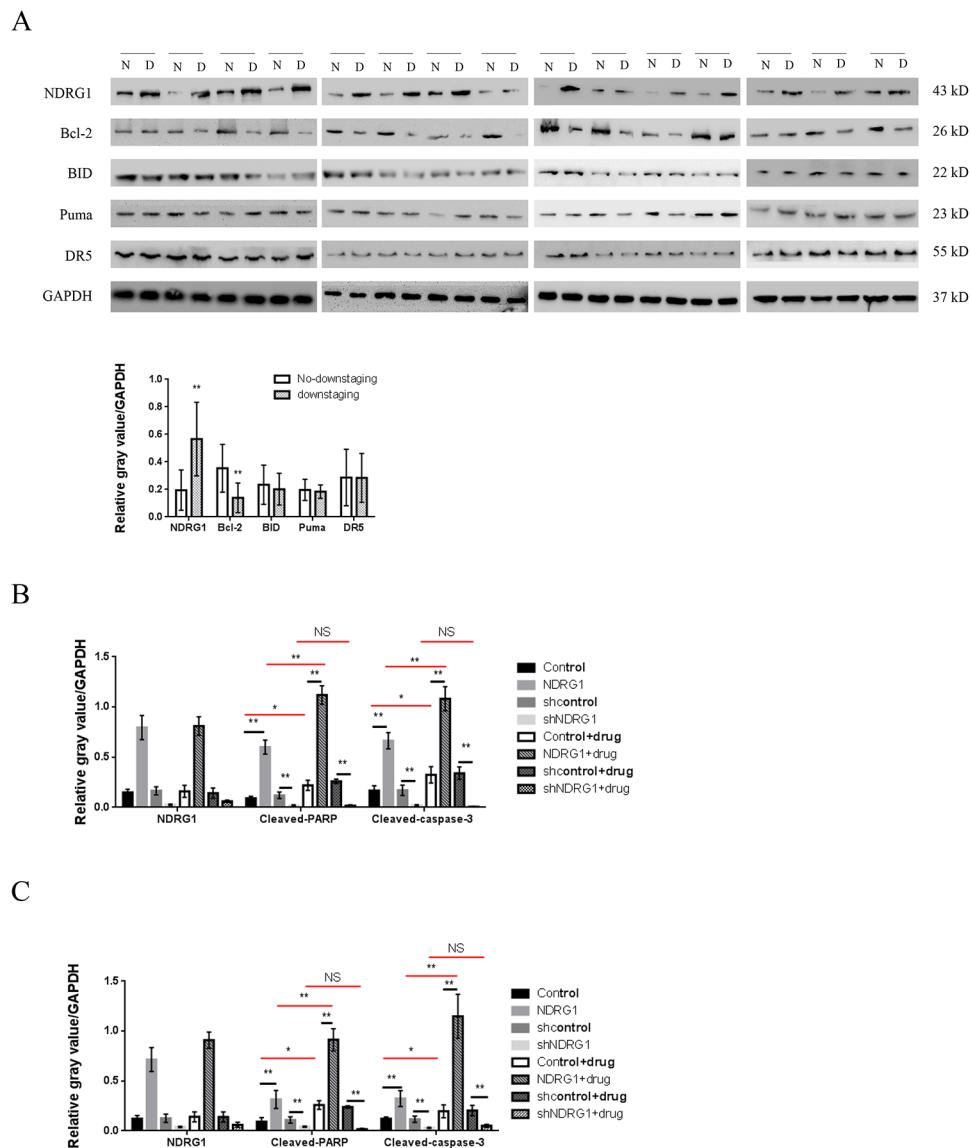
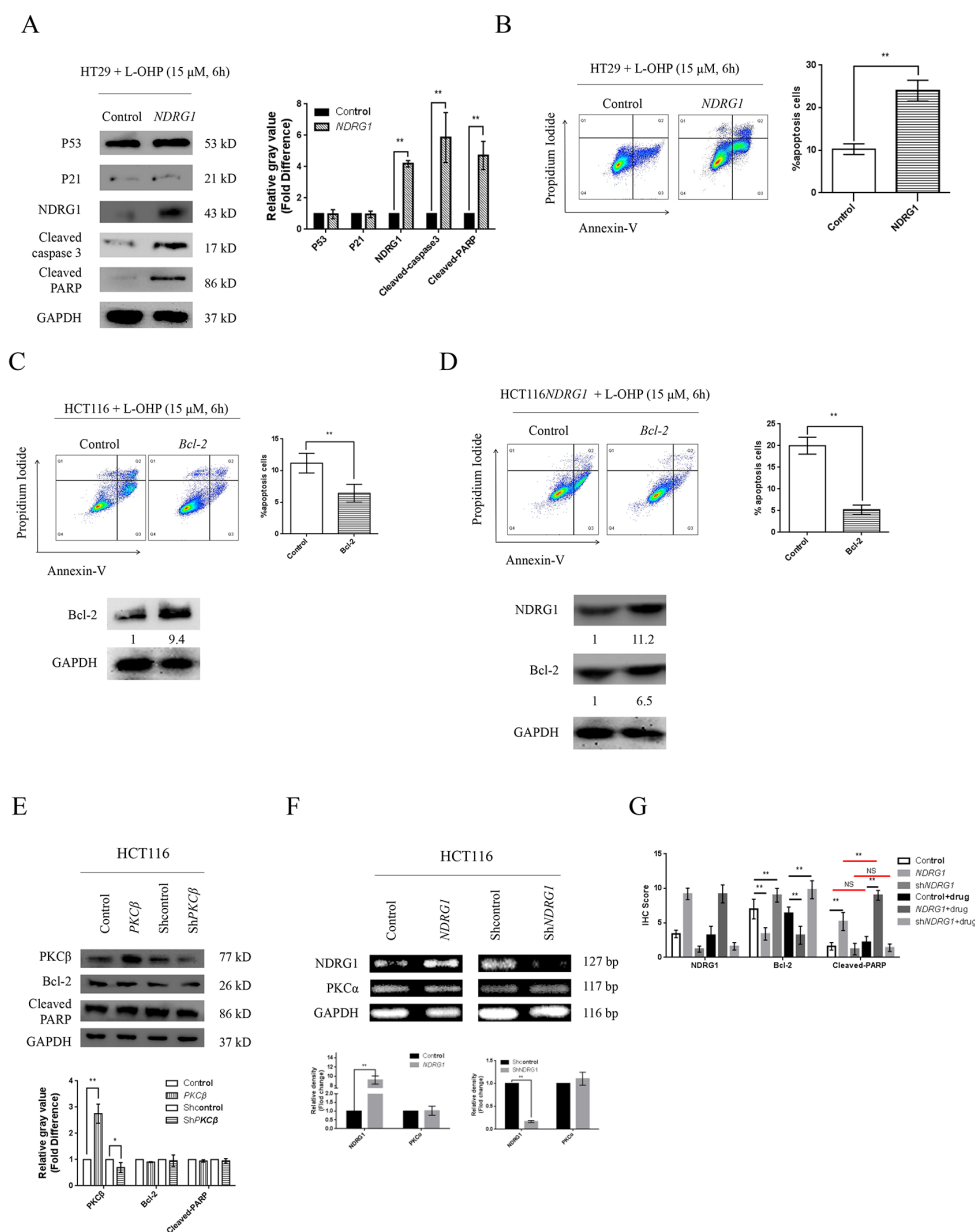


# N-myc downstream-regulated gene 1 promotes oxaliplatin-triggered apoptosis in colorectal cancer cells via enhancing the ubiquitination of Bcl-2

## SUPPLEMENTARY FIGURES AND TABLE



**Supplementary Figure 1:** (A) Western blots showing protein levels of several apoptosis regulating factors namely NDRG1, Bcl-2, Puma, BID, and death receptor 5 (DR5) in selected patients from no-downstaging and downstaging group respectively. (B, C) Quantitative analysis of protein levels showed in Figure. 2C (B) and 2D (C). Red line indicated comparison between drug-treated and -untreated groups. Data were from three independent experiments. Bars indicated SD. NS, not significant, \*,  $P < 0.05$ , \*\*,  $P < 0.01$ .



**Supplementary Figure 2:** (A) *NDRG1* restored the response of HT29 cells to oxaliplatin. After 6 hours incubation with oxaliplatin (15 $\mu$ M), apoptosis in HT29*NDRG1* cells and control cells were analyzed with western blots. Low level of P21 was an evidence of defective *TP53* gene in HT29 cells. (B) Apoptotic cell numbers in HT29 *NDRG1* derivatives were analyzed by flow cytometry assay with Annexin V/PI. (C, D) Large amounts (10 $\mu$ g) of *Bcl-2* constructs or control vector were transfected into HCT116 cells (C) or HCT116-*NDRG1* (D) cells. Transfects were then cultured in medium containing oxaliplatin (15 $\mu$ M). Apoptosis were analyzed with flow cytometry assay. The successful transfection of *Bcl-2* and *NDRG1* were confirmed by western blots. Densitometry showed relative protein expression normalized for GAPDH. (E) *PKC $\beta$*  status had no effect on the stability of *Bcl-2* or apoptosis. HCT116 cells were transfected with *PKC $\beta$* , sh*PKC $\beta$*  or control vector. Protein level of *Bcl-2* was then detected with western blot. (F) *NDRG1* was unable to modulate mRNA level of *PKC $\alpha$* . After transfection of *NDRG1* constructs or control vector (10 $\mu$ g), total RNA was extracted and subjected to RT-PCR. (G) Quantitative analysis of IHC scores of Figure. 6D. Red line indicated comparison between drug-treated and -untreated groups. Histograms showed mean values from three independent experiments; bars indicated SD; NS, not significant, \*,  $P < 0.05$ , \*\*,  $P < 0.01$ .

**Supplementary Table 1: Detailed information of patients.**

**See Supplementary File 1**