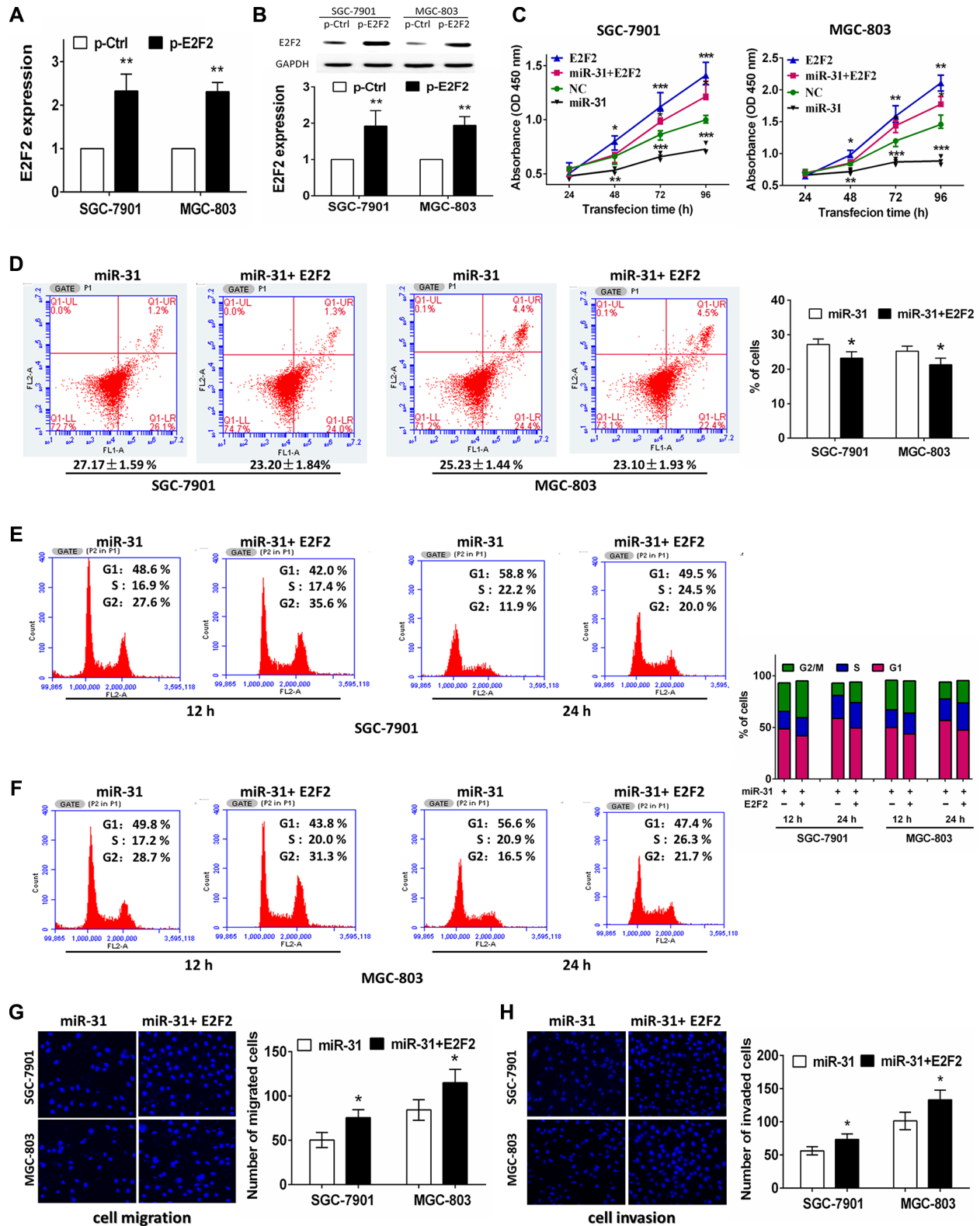


# Downregulated miR-31 level associates with poor prognosis of gastric cancer and its restoration suppresses tumor cell malignant phenotypes by inhibiting E2F2

## Supplementary Materials



**Supplementary Figure S1: Effects of E2F2 restoration on gastric cancer SGC-7901 and MGC-803 cells.** Tumor cells were transiently transfected with miR-31 mimics or miR-31 mimics and pWSLV-01-E2F2 plasmid and then subjected to different assays. (A) RT-PCR. Level of E2F2 mRNA was assessed by RT-PCR after transfection with pWSLV control or pWSLV-01-E2F2 into gastric cancer cells. (B) Western blot. Level of E2F2 protein was assessed after transfection with pWSLV control or pWSLV-01-E2F2 into gastric cancer cells. The relative protein levels were normalized to GAPDH and data were presented as mean  $\pm$  sd. (C) Cell viability CCK8 assay. Cell viability was assessed after 24, 48, 72 and 96 h transfection with miR-31 mimics or miR-31 mimics and pWSLV-01-E2F2 plasmid into gastric cancer cells. Data were presented as mean  $\pm$  sd of three independent experiments.  $*p < 0.05$ ,  $**p < 0.01$  and  $***p < 0.001$ . (D) Apoptosis assay. Level of apoptosis was assessed after 48 h transfection with miR-31 mimics or miR-31 mimics and pWSLV-01-E2F2 plasmid into gastric cancer cells. Data were presented as mean  $\pm$  sd of three independent experiments with duplicated samples.  $*p < 0.05$ . (E–F) Flow cytometric cell cycle assay. Cell cycle distribution was analyzed additional culture of 12 and 24 h after 48 h transfection with miR-31 mimics or miR-31 mimics and pWSLV-01-E2F2 plasmid into gastric cancer cells. (G) Tumor cell migration assay. Fluorescent images ( $\times 200$ ) (left) and quantification (right) of migration level of SGC-7901 and MGC-803 cells after 24 h transfection with miR-31 mimics or miR-31 mimics and pWSLV-01-E2F2 plasmid. Data were presented as mean  $\pm$  sd,  $*p < 0.05$ . (H) Tumor cell invasion assay. Fluorescent images (left) and quantification (right) of invasion level of SGC-7901 and MGC-803 cells after 24 h transfection with miR-31 mimics or miR-31 mimics and pWSLV-01-E2F2 plasmid. Data were presented as mean  $\pm$  sd,  $*p < 0.05$ .