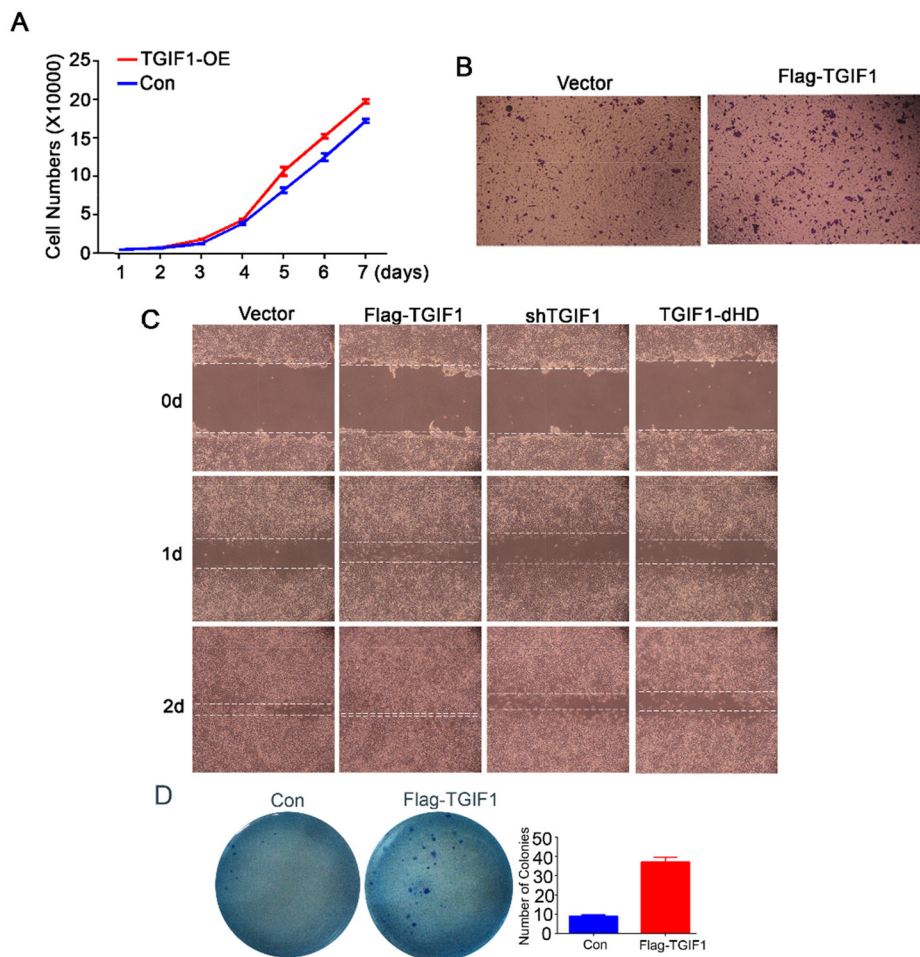
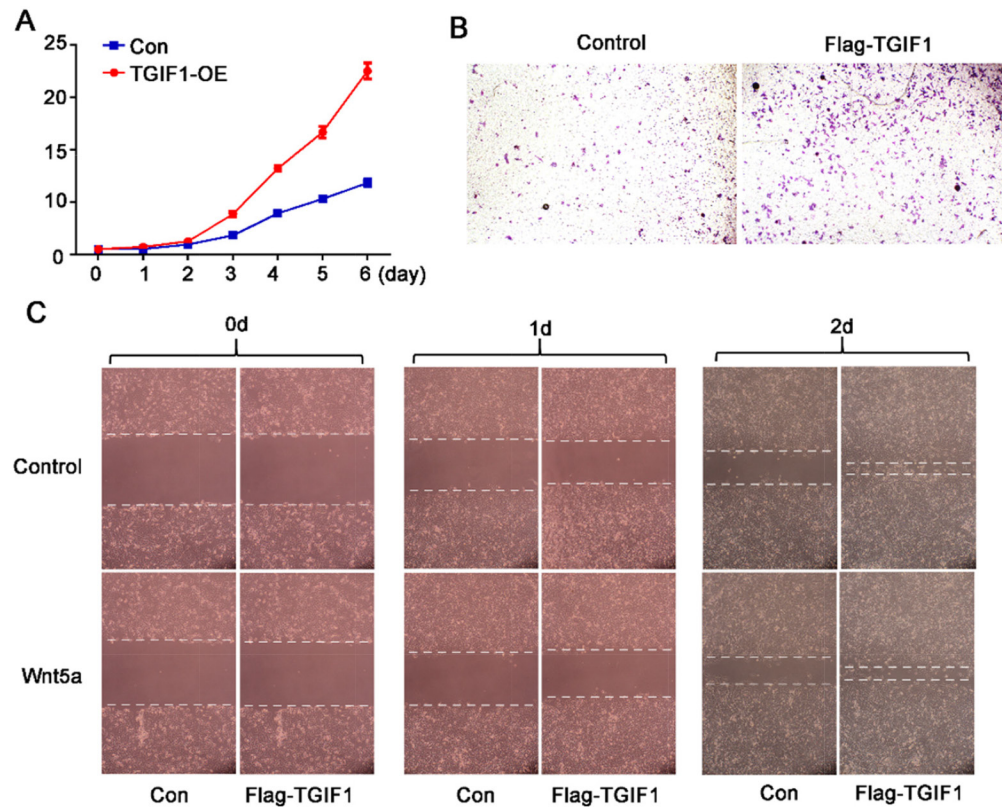


# TGFβ induced factor homeobox 1 promotes colorectal cancer development through activating Wnt/β-catenin signaling

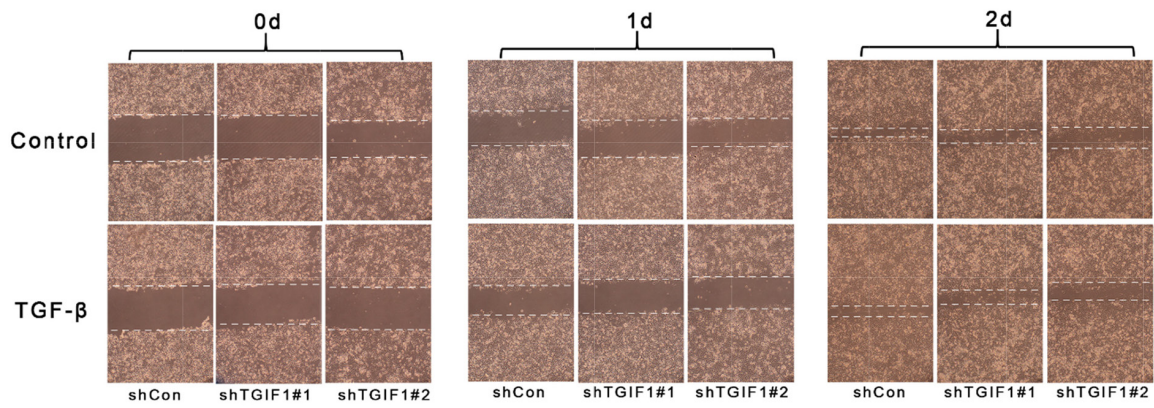
## SUPPLEMENTARY MATERIALS



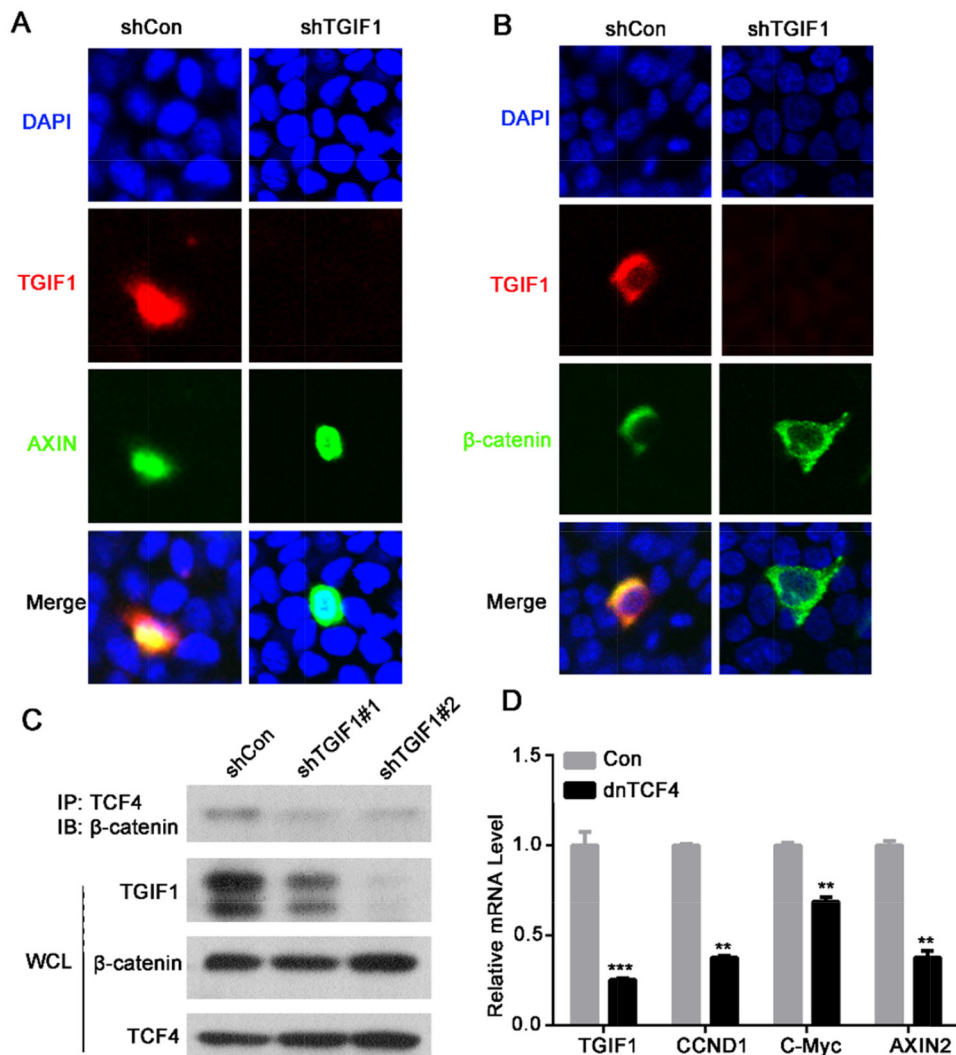
**Supplementary Figure 1:** (A) Growth curve of control and TGIF1 overexpressed LoVo cells. (B) Transwell assay using control and TGIF1 knockdown cells. After seeding cells for 24 hours, the number of migrated cells was quantified after staining with gentian violet. (C) Wound healing assay in control and TGIF1 overexpression, TGIF1 knockdown, and deletion of TGIF1 homeodomain cells. Cells were photographed every 24h after scratching. (D) Soft agar colony formation assay using control and TGIF1 overexpressed cells. Images were obtained after staining with Giemsa.



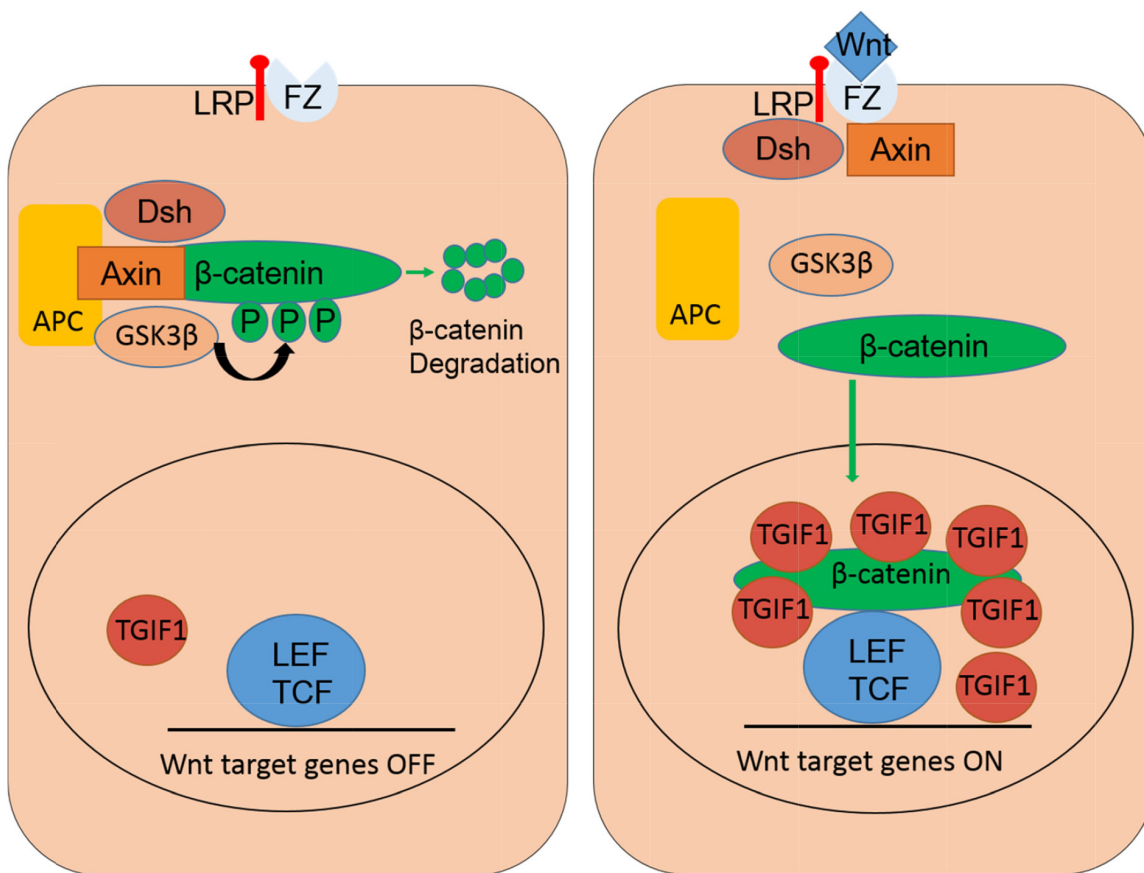
**Supplementary Figure 2:** (A) Growth curve of control and TGIF1 overexpressed CaCo-2 cells. (B) Transwell assay using control and TGIF1 overexpressed CaCo-2 cancer cells. After seeding cells for 24 hours, the number of migrated cells was quantified after staining with gentian violet. (C) Wound healing assay in control and TGIF1 overexpressed CaCo-2 cancer cells with Wnt5a treatment. Cells were photographed every 24h after scratching.



**Supplementary Figure 3: Wound healing assay in control and TGIF1 knockdown LoVo cells under TGF- $\beta$  (100pM).** Cells were photographed every 24h after scratching.



**Supplementary Figure 4:** (A-B) LoVo cells were harvested for anti-TGIF1/AXIN/ $\beta$ -Catenin immunostaining. (C) LoVo cell lysates were precipitated with anti-TCF4 antibody, followed by anti- $\beta$ -catenin immunoblotting (IB) to detect the interaction between  $\beta$ -catenin and TCF4. (D) mRNA levels of Wnt target genes (*CCND1*, *c-Myc*, *AXIN2*) were examined by qRT-PCR in control and dominant TCF4 HCT116 cells.



**Supplementary Figure 5: (Left) In the absence of Wnt stimulation, β-catenin levels are kept at a minimum through the destruction complex. (Right) In the presence of Wnt stimulation, the destruction complex is destabilized and TGIF1 upregulated, and β-catenin accumulates in the nucleus to activate transcription of Tcf target genes. In addition, TGIF1 could activate Wnt signaling through direct DNA binding, but more evidence waits to be provided.**