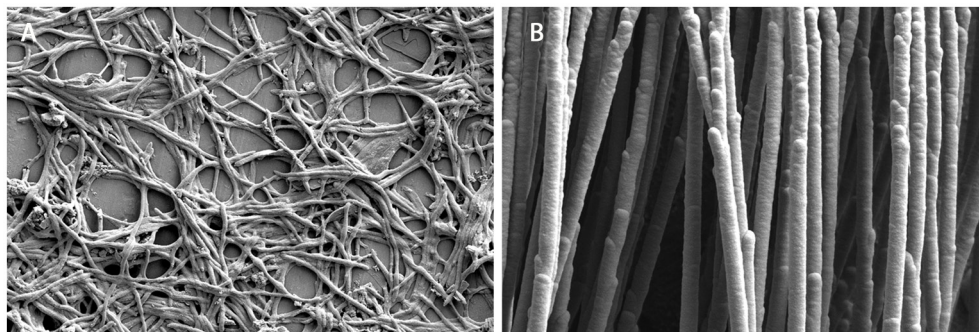
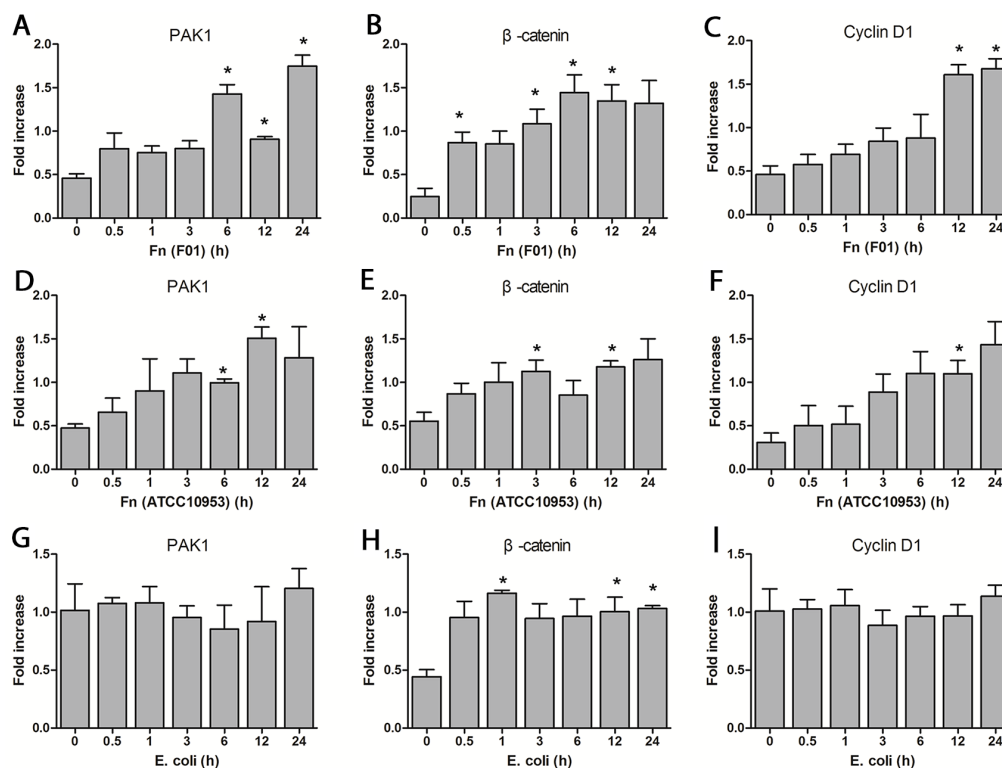


Invasive *Fusobacterium nucleatum* activates beta-catenin signaling in colorectal cancer via a TLR4/P-PAK1 cascade

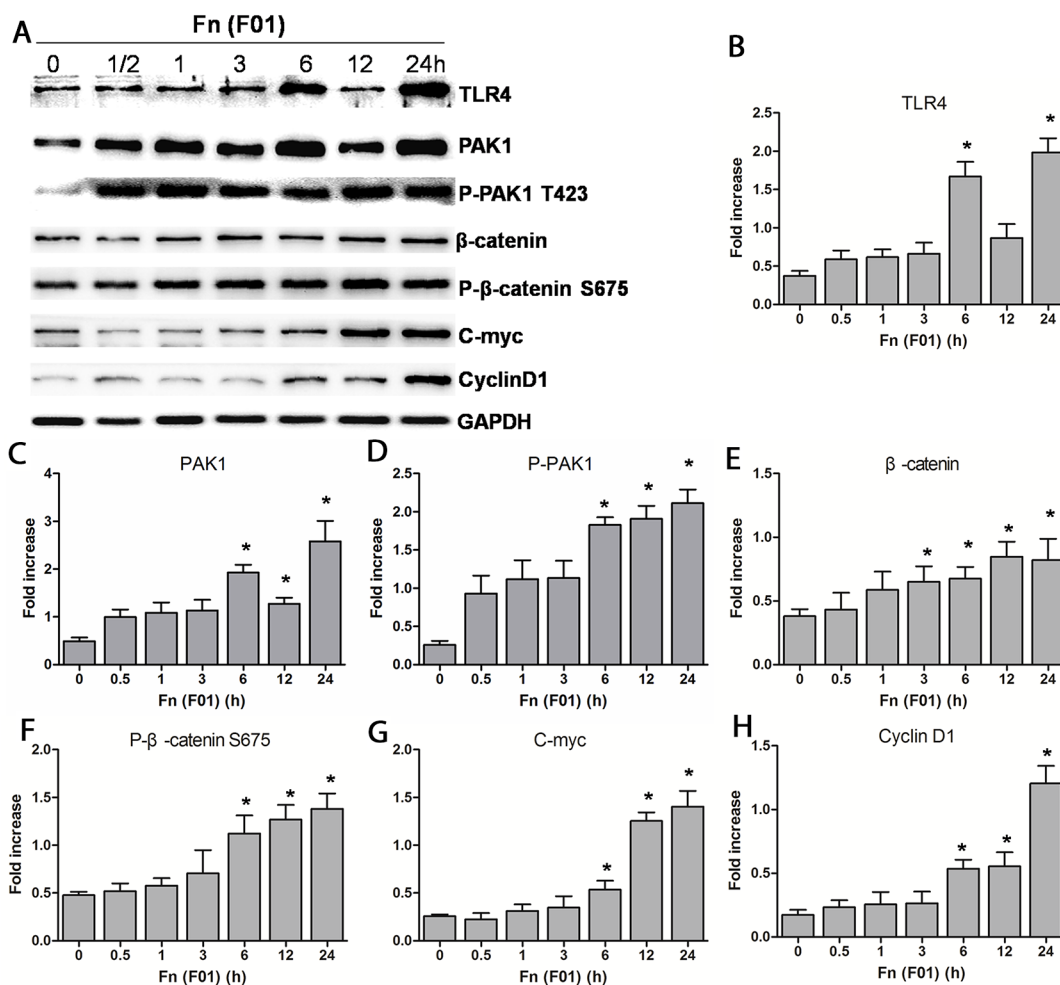
SUPPLEMENTARY FIGURES



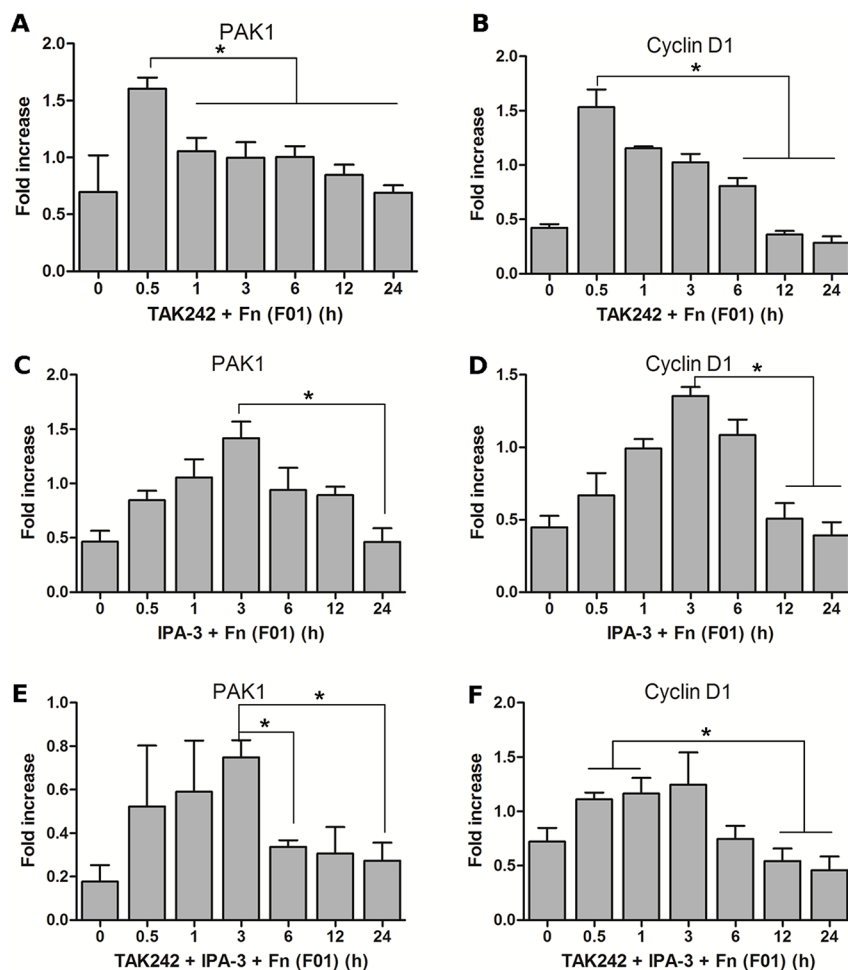
Supplementary Figure 1: Scanning electron microscopy shows the shape of cultured *Fn*. The strain was isolated from frozen tumor sections of a right-sided colon cancer and named *F01*. (A) 5,000 \times magnification; (B) 20,000 \times magnification.



Supplementary Figure 2: *Fn* activates the β -catenin signaling pathway possibly through the TLR4/P-PAK1/P- β -catenin S675 cascade. (A-C) Western blots showing that the levels of PAK1, β -catenin and Cyclin D1 gradually increase when SW480 cells are challenged with *Fn* (*F01*) over time. (D-F) The PAK1, β -catenin and Cyclin D1 levels also gradually increase when SW480 cells are challenged with *Fn* (*ATCC10953*) over time. (G-I) The PAK1 and Cyclin D1 levels do not significantly increase when SW480 cells are challenged with *E. coli* for increasing time periods, although the protein abundance of total β -catenin significantly increases. Bar diagrams represent the results obtained after densitometric scanning from three different experiments. Bars represent the mean \pm SD. *, $P < 0.05$, as compared with control group (0 h).



Supplementary Figure 3: *Fn (F01)* activates the β-catenin signaling pathway in Caco-2 cells through the TLR4/P-PAK1/P-β-catenin S675 cascade. (A) Western blots showing that the protein levels of TLR4, total PAK1, P-PAK1, total β-catenin, P-β-catenin S675, C-myc and Cyclin D1 increase with similar trends when Caco-2 cells are challenged with *Fn (F01)* over increasing time periods. (B-H) Bar diagrams represent the results obtained after densitometric scanning from three different experiments. Bars represent the mean ± SD. *, $P < 0.05$, as compared with control group (0 h).



Supplementary Figure 4: Activation of the β -catenin signaling pathway by *Fn* (F01) can be inhibited by both the TLR4 inhibitor (TAK-242) and PAK1 inhibitor (IPA-3). (A, B) Western blots showing that PAK1 and Cyclin D1 levels significantly decrease when SW480 cells are treated with TAK-242 before *Fn* (F01) challenge. (C, D) Western blots showing that PAK1 and Cyclin D1 levels significantly decrease when SW480 cells are treated with IPA-3 before *Fn* (F01) challenge. (E, F) Western blots showing that PAK1 and Cyclin D1 levels significantly decrease when SW480 cells are treated with both TAK-242 and IPA-3 before *Fn* (F01) challenge. Bar diagrams represent the results obtained after densitometric scanning from three different experiments. Bars represent the mean \pm SD. *, $P < 0.05$.