FH535 Inhibits Proliferation and Motility of Colon Cancer Cells by Targeting Wnt/β-catenin Signaling Pathway

Supplementary file

Supplementary methods

Dual luciferase reporter assay

Dual luciferase reporter assay was carried out using the reporter plasmid TOPFlash (TCF reporter plasmid, Addgene). The internal control plasmid pRL-SV40 contains the *Renilla* luciferase gene. Cells were transiently co-transfected with TOPFlash / pRL-SV40 vectors for 6 hours using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. The medium was renewed and FH535 was added. After 24 hours of treatment, cell lysates were subjected to the dual luciferase reporter assay using Dual-Luciferase® Reporter Assay System (Promega). Thereafter, luciferase activity was measured using a luminometer. Relative luciferase activity was calculated as the ratio of firefly luciferase activity over *Renilla* luciferase activity.

Table S1 Expression of CSC markers at mRNA level in HT29 and SW480 cells. Expression normalized to *GAPDH*.

CSC marker	means-HT29 (log2 /GAPDH)	means-SW480 (log2 /GAPDH)	Fold change (log2 SW480/HT29)	p value
CD24	0.92	-4.76	-5.68	<0.001
CD44	-2.98	-4.79	-1.81	<0.001
CD133	-3.75	-15.74	-11.99	<0.001

Table S2 See excel file Supplementary Table S2. Pathway enrichment analysis of DEGs based on KEGG database. Top 20 pathways ranked by Q value were shown.

Table S3 See excel file Supplementary Table S3. GO analysis of DEGs. GO terms with P value <0.05 were shown.

Table S4 List of genes of interest in the present study and their differential expression between FH535-treated versus control HT29 based on RNA-seq. Gene *PROM1* encodes CD133, CTNNB encodes beta-cateinin, *CCND1*encodes cyclin D1, *BIRC5* encodes survivin.

GeneID	**Gene symbol**	**Means-Control**	**Means-FH535**	**log2 Ratio (FH535/Control)**	**Divergence probability**
100133941	CD24	428.09	193.14	-1.15	0.92
960	CD44	119.94	61.90	-0.95	0.89
8842	PROM1	35.56	9.90	-1.85	0.94
1499	CTNNB1	60.13	58.07	-0.05	0.30
51176	LEF1	0.11	0.01	-3.46	0.26
8313	AXIN2	20.79	10.68	-0.96	0.88
595	CCND1	30.09	19.63	-0.62	0.85
332	BIRC5	18.55	9.79	-0.92	0.87
4316	MMP7	71.54	21.84	-1.71	0.94

Supplementary figures

Figure S1

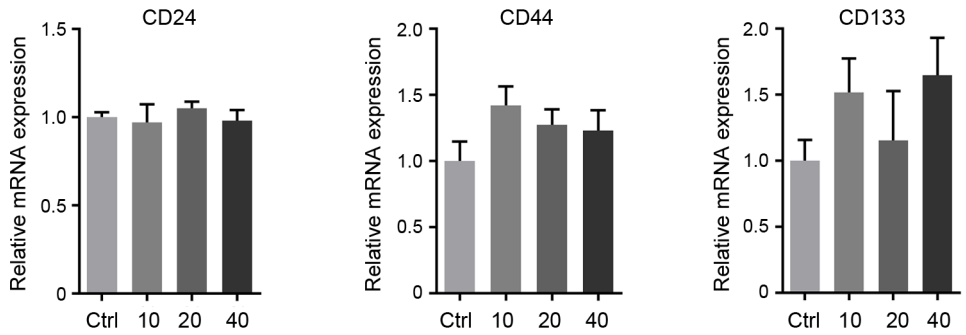

Figure S1. The effect of FH535 on mRNA expression of CSC markers in SW480.

Results were determined by RT-qPCR, normalized to GAPDH. Ctrl: Control. Units of FH535 doses were μM.

Figure S2

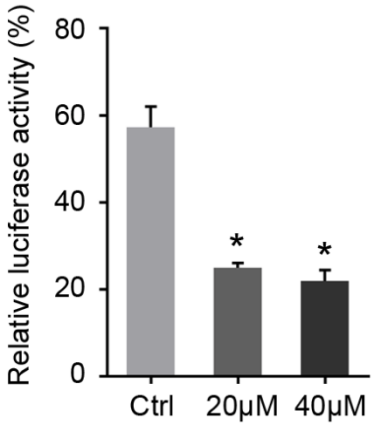

Figure S2. FH535 suppresses TCF-dependent transcription in SW480 cells. TCF-dependent transcription determined by dual luciferase reporter assay using the reporter plasmid TOPFlash. SW480 cells were treated with DMSO, 20 or 40 μM FH535. data presented as relative luciferase activity. *$p < 0.05$ verses control. Ctrl: Control.

Figure S3

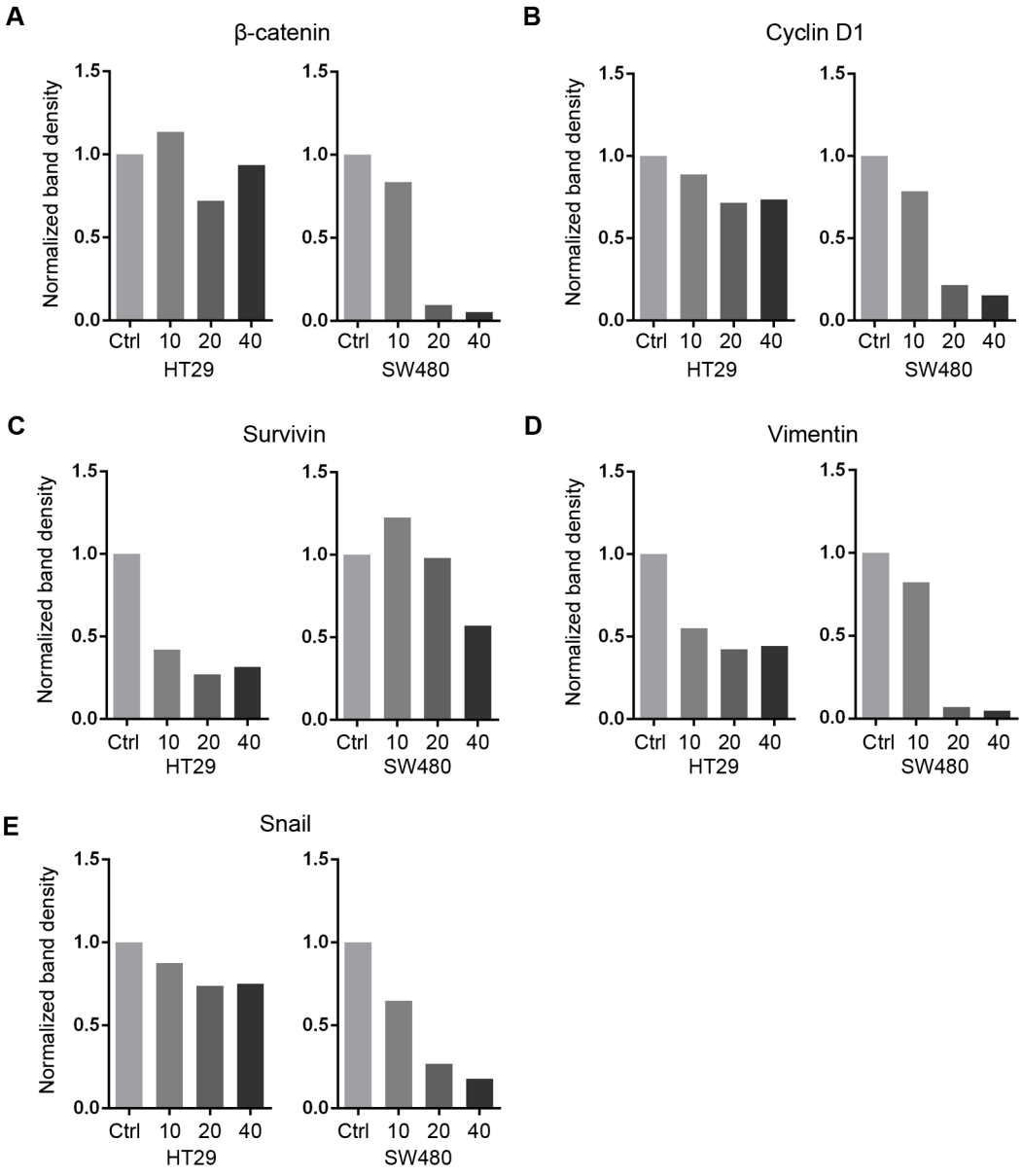

Figure S3. FH535 downregulated wnt/β-catenin pathway target genes at proten level. Semi-quantification of positive bands in Western-blotting (images presented in Figure 4). Ctrl: Control. Units of FH535 doses were μM.

Figure S4

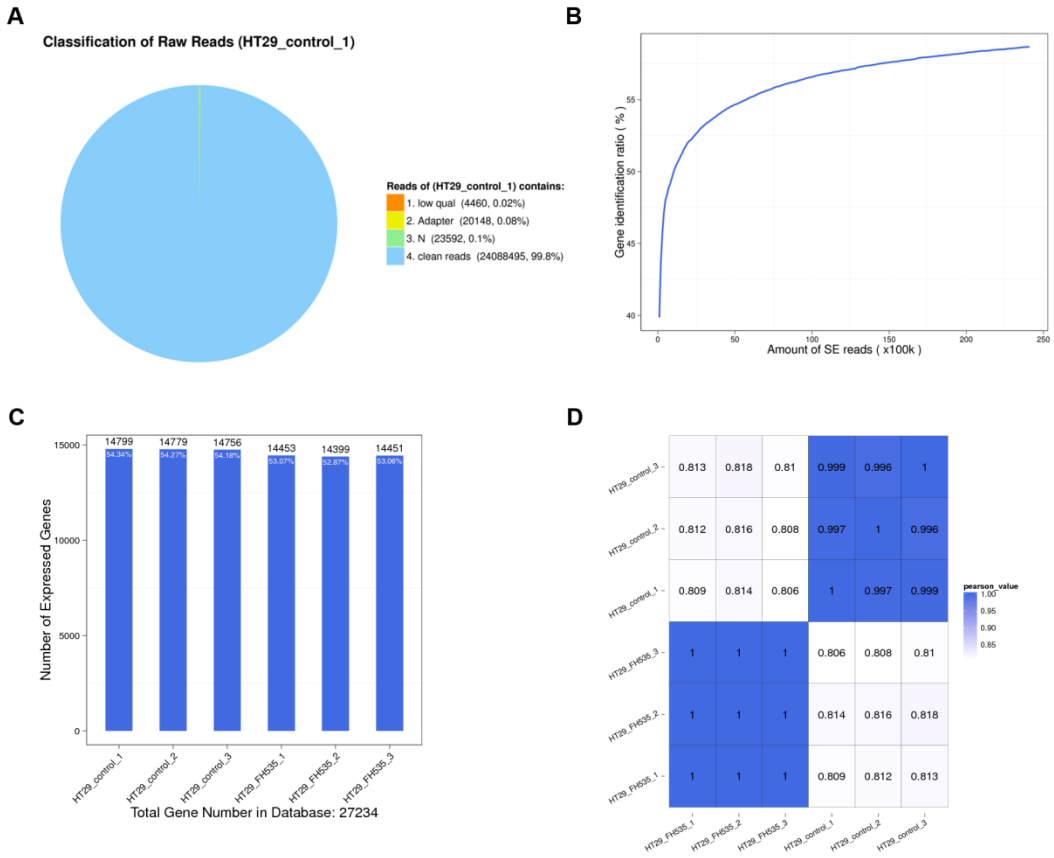

Figure S4. Quality control and quantitative analysis of RNA sequencing data. (A) Representative quality control statistics of raw reads (sample HT29_Control_1). (B) Representative sequencing saturation analysis (sample HT29_Control_1). X-axis shows the number of clean reads, Y-axis shows the ratio of identified genes to total genes in reference database. (C) Bar graph showing number of identified genes in each sample. (D) Heatmap showing correlation coefficient values across samples.

Figure S5

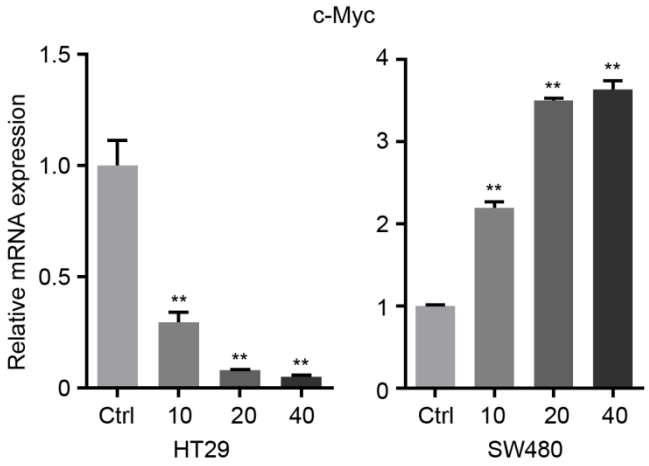

Figure S5. The effect of FH535 on c-Myc mRNA expression in HT29 and SW480.

Results were determined by RT-qPCR, normalized to GAPDH. $^{**}p < 0.01$. Ctrl: Control. Units of FH535 doses were μM.