

PIK3C3 Inhibition Promotes Sensitivity to Colon Cancer Therapy by Inhibiting Cancer Stem Cells

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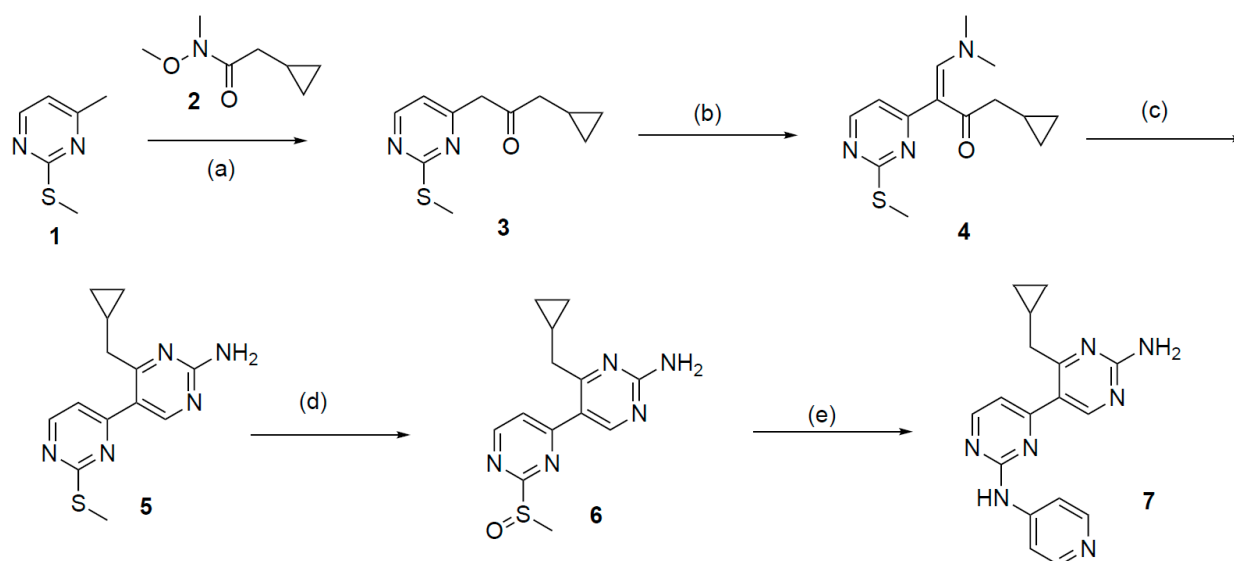


Figure S1. Scheme-1 of the synthesis of 36-077: Scheme-1, Reagents and conditions. (a) LDA (2.0 M in THF), THF, 2, -10°C; (b) DMF-DMA, 80°C, 2 h, 80%; (c) N-methylguanine, K₂CO₃ DMF, 120°C, 3 h, rt, 16 h, 72%; (d) m-CPBA, CH₂Cl₂, 10°C, 45 mins, 67%; (e) LiHMDS, 4-aminopyridine, THF, -10°C - > 1.5 h, 6%.

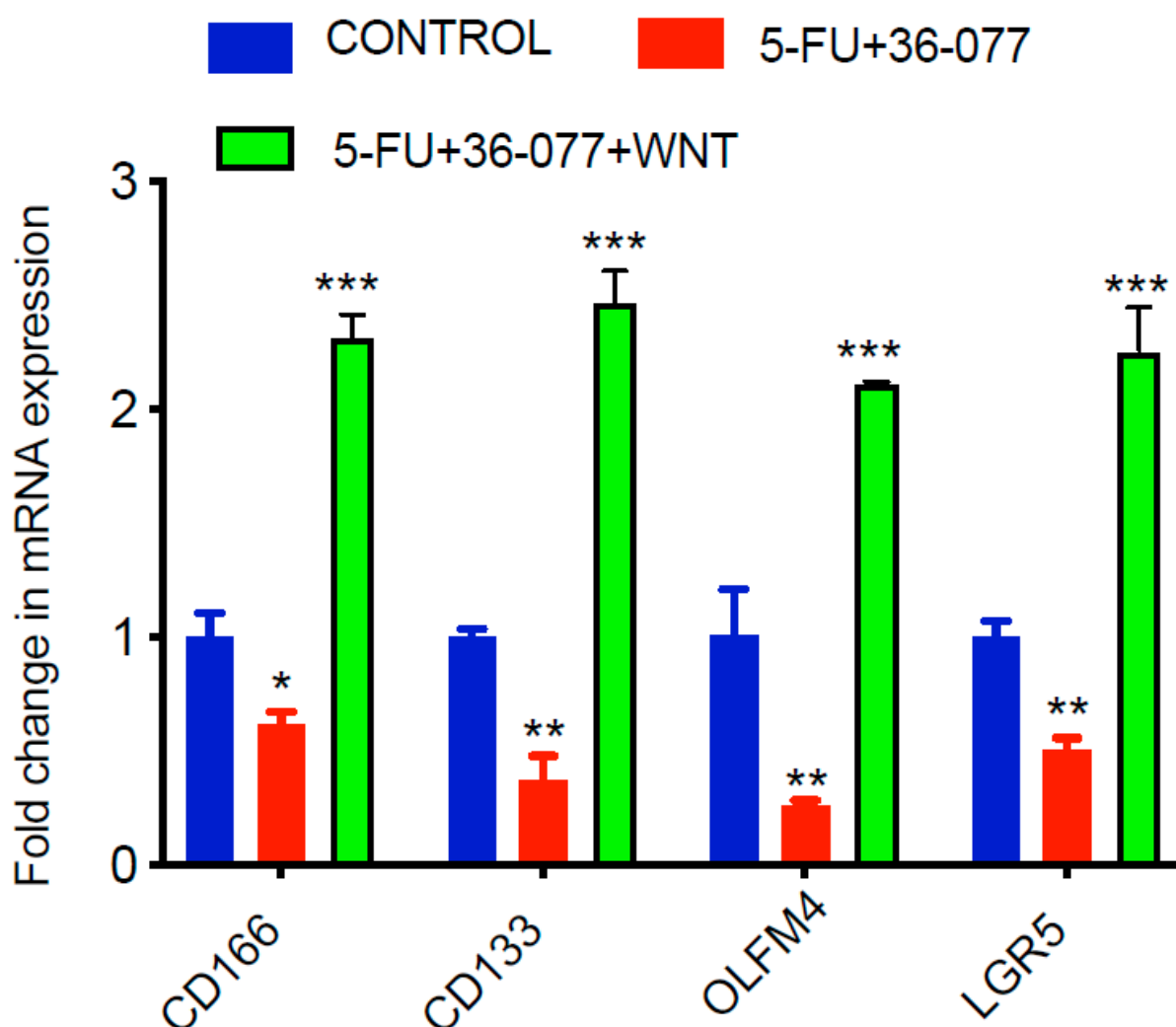


Figure S2. Wnt-signaling activation protects cancer stem cells from the co-treatment effects of 5-FU and 36-077: Real time qPCR for expression of stem cells markers using total RNA isolated from the HCT116 cells treated with 5-FU and 36-077 in combination with or without WNT3A. Data is presented as mean+sem. Statistical significance was determined by 1-way ANOVA and post hoc Tukey's test for pairwise comparison. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

Table S1. List of reagents, antibodies and kits.

S.N.	Antibody/Kit	Company	Catalog Number
1	jetPRIME transfection Reagent	Polyplus transfection	114-07
2	Cleaved caspase 3 rabbit anti-body	Cell signaling Technology	9661S
3	Anti-LC3 rabbit antibody	Sigma	L8918-25UL
4	Anti-p62/SQSTM1 rabbit anti-body	Sigma	P0067-25UL
5	β -Actin mouse antibody	Santa Cruz technology	sc-47778
6	Cyclin D1 Monoclonal rabbit anti-body	Cell signaling Technology	55506
7	Corning Matrigel Matrix	Corning	356255
8	β -Catenin mouse monoclonal antibody	BD Biosciences	610153
9	Phospho- β -Catenin (Ser33/37) monoclonal rabbit antibody	Cell signaling Technology	2009S

10	GSK-3 β (D5C5Z) rabbit monoclonal antibody	Cell signaling Technology	12456
11	Phospho-GSK-3 β (Ser9) (D85E12) monoclonal rabbit antibody	Cell signaling Technology	5558
12	Survivin (71G4B7) Rabbit monoclonal antibody	Cell signaling Technology	2808
13	OLFM4 (D1E4M) XP Rabbit monoclonal antibody	Cell signaling Technology	14369
14	Dual Luciferase assay kit	Promega	E2920

Table S2. Stem cells maker primer.

S.N	Primer	5'-----Sequence-----3'
1	H_ β -ACTIN FP	CCA AGC ACA ATG AAG ATC AA
2	H_ β -ACTIN RP	ACA TCT GCT GGA AGG TGG AC
3	H_OLFM4FP	TGC CAT TCG CCG AGA AAT C
4	H_OLFM4RP	GGA CGA CAG GGG TGT TTT GAT
5	H_CD133FP	GGT GCT GTT CAT GTT CTC CA
6	H_CD133FP	ACC GAC TGA GAC CCA ACA TC
7	H_LGR5FP	CTC CCA GGT CTG GTG TGT TG
8	H_LGR5RP	GCT CGC AAT GAC AGT GTG TG
9	H_CD166FP	TAG CAG GAA TGC AAC TGT GG
10	H_CD166RP	CGC AGA CTA GTT CCA GCA
11	M_ β -ACTIN FP	CCA GAG CAA GAG AGG TAT CC
12	M_ β -ACTIN RP	CTG TGG TGG TGA AGC TGT AG
13	M_CD133FP	GAA AAG TTG CTC TGC GAA CC
14	M_CD133RP	TCT CAA GCT GAA AAG CAG CA
15	M_CD166FP	TTTGGCGCAGATGGAAGTGA
16	M_CD166RP	TGTGCGTTGAAAGGAGGTG
17	M_LGR5FP	CTA CTT GAC TTT GAG GAA GAC C
18	M_LGR5RP	AGG AAA GCG CCA GTA CTG C