Supplementary Materials: The *MYC* 3′ Wnt-Responsive Element Drives Oncogenic *MYC* Expression in Human Colorectal Cancer Cells

Sherri A. Rennoll, Melanie A. Eshelman, Wesley M. Raup-Konsavage, Yuka Imamura Kawasawa and Gregory S. Yochum

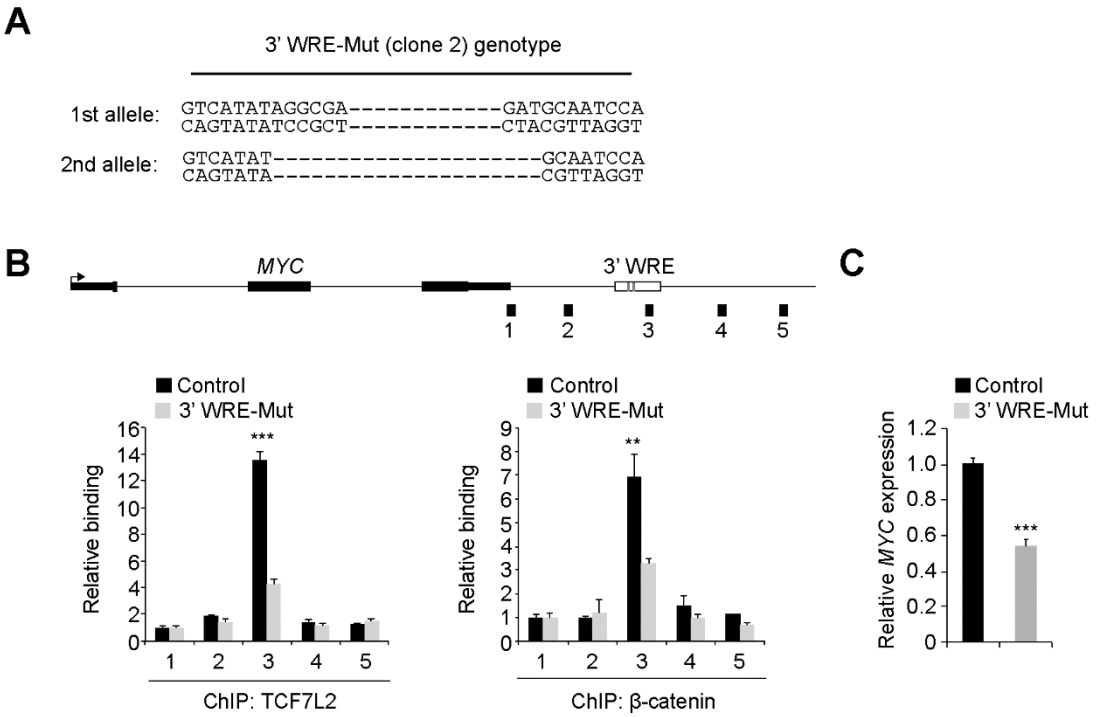

Figure S1. TCF7L2/β-catenin binding to the *MYC* 3′ WRE and *MYC* expression are reduced in a second 3′ WRE-Mut clonal HCT116 cell line. (**A**) Sequences of the targeted *MYC* 3′ WRE alleles in the second clone. Refer to Figure 1 for the wild-type sequence; (**B**) qPCR analysis of DNA fragments precipitated with anti-TCF7L2 or anti-β-catenin antibodies in ChIP assays conducted in control and 3′ WRE-Mut HCT116 cells. The position of the PCR amplicons used to detect binding (1-5) are indicated on the diagram of the *MYC* locus above. The signals are normalized to levels detected with amplicon 1; (**C**) qRT-PCR analysis of *MYC* transcript levels detected in control and 3′ WRE-Mut cells. The data is normalized to *GAPDH* levels. Error bars are ±SEM (** $p < 0.01$, *** $p < 0.001$).

MYC 3′ TBE1 CRISPR target sequence: GTCATATAGGCGAATTTCAAAGG

off-target 1	chr8:138982738	TACATATAGGAGAATTTCAAAGG	3 mismatches [1, 2, 11]
off-target 2	chr4:168449180	TTCATATTGGCTAATTTCAAAAG	3 mismatches [1, 8, 12]
off-target 3	chr5: 3826037	TTCATTTAGGAGAATTTCAATAG	3 mismatches [1, 6, 11]
off-target 4	chr10: 26930866	TTCTTATGGGTGAATTTCAATGG	4 mismatches [1, 4, 8, 11]
off-target 5	chr9: 106293769	TTTAGATAGGTGAATTTCAACAG	4 mismatches [1, 3, 5, 11]
off-target 6	chr21: 32202491	GGCACATGGGTGAATTTCAAGAG	4 mismatches [2, 5, 8, 11]
off-target 7	chr12: 77224237	GCCATATGGAAGAATTTCAATAG	4 mismatches [2, 8, 10, 11]
off-target 8	chr1: 246908861	GGGATATAGCTGAATTTCAAGGG	4 mismatches [2, 3, 10, 11]
off-target 9	chr7: 128780931	GAAATATGGGCCAATTTCAACAG	4 mismatches [2, 3, 8, 12]
off-target 10	chr5: 85292377	ATTATAGAGGAGAATTTCAAAAG	4 mismatches [1, 3, 7, 11]
FAM112A	chr20: 42355297	ATCATAAGGGCAAATTTCAAAGG	4 mismatches [1, 7, 8, 12]
GNA13	chr17: 63007955	TTCATATAGTGGTATTTCAAAAG	4 mismatches [1, 10, 11, 13]
KCNH7	chr2: 163256716	GTCATCTTTGAGAATTTCAATGG	4 mismatches [6, 8, 9, 11]
MYO1E	chr15: 59664763	GTCTTCTGGGCGAACTTCAAAGG	4 mismatches [4, 6, 8, 15]
ALDH1L1	chr3: 125850152	GCCATATAGGAGAACTCCAAGGG	4 mismatches [2, 11, 15, 17]
CSMD3	chr8: 113237073	GTCATACATGGGATTTTCAAAAG	4 mismatches [7, 9, 11, 14]

Figure S2. Insertions or deletions (indels) are absent at the top predicted off-targets in 3′ WRE-Mut cells. PAGE analysis of PCR products amplified from genomic DNA isolated from parental HCT116 (control) or 3′ WRE-Mut cells using primers that anneal to regions flanking the putative off-target sites. Listed are the targets analyzed and their chromosomal position. Nucleotides that are not conserved in the *MYC* 3′ WRE CRISPR target sequence are in red. The PAM sequence is underlined.