Supplementary Figure Legend

Supplementary Figure 1. Effect of EZH2 knockdown on wound healing, spheroid formation and stem cell markers expression. Ai. Real-time PCR analysis of total RNA isolated from uninfected or CR-infected HEK-293T cells with EZH2 specific primers (*p<0.05; n = 5 independent experiments). **Aii.** Relative levels of EZH2 following EZH2 knockdown. CR infected HEK-293T cells were transfected with control siRNA (C) or EZH2-specific siRNAs (siEZH2a, siEZH2b) and total RNA was subjected to real-time PCR (*p<0.05; n = 5 independent experiments). **B.** Western blot showing effect of EZH2 knockdown on EZH2 and β-catenin levels, respectively. **C. Effect of EZH2 depletion on wound healing**. HEK-293T cells were either transfected with non-targeting control (Cont.) siRNA or siRNA specific for EZH2 for 24h followed by wound-healing assay. **D. Effect of EZH2 depletion on spheroid formation.** HEK-293T or HCT116 cells were either transfected with non-targeting control (Cont.) siRNA or siRNAs specific for EZH2 or untreated (Cont.) and treated with sodium butyrate (NaB) for 24h followed by spheroid assay. A representative photomicrograph showing spheroid assay in duplicates (n = 5 independent experiments). **E-G.** Effect of EZH2 knockdown on expression of cancer stem cell markers. Real-time PCR for CD44 (**E**), Dclk1 (**F**) and Lgr5 (**G**) expression in total RNA isolated from HEK-293 cells transfected with control (C) or EZH2-specific siRNAs (siEZH2a, EZH2b) (*p<0.05; n = 3 independent experiments).

Supplementary Figure 2. Effect of EZH2 knockdown on expression of cancer stem cell markers. HCT116 colon cancer cells were transfected with either control siRNA or EZH2-specific siRNAs (siEZH2a, EZH2b) and the levels of EZH2 (**A**), CD44 (**B**), Dclk1 (**C**) and Lgr5 (**D**) expression were determined via real-time PCR (*p<0.05; n = = 3 independent experiments).

Supplementary Figure 3. Effect of wild type and mutant CR infection on Wnt antagonists and EZH2 expression. A. Relative levels of EZH2 and WIF1, measured in total extract from HEK-293 cells transfected with either control siRNA (Cont-siRNA) or EZH2-specific siRNA (EZH2-siRNA). **B-C. Effect of wild type or mutant CR (ΔescV) infection on expression of Wnt antagonists in HEK-293 cells.** HEK-293 cells were either uninfected or infected with either wild type or ΔescV mutant for 3h, washed with culture medium to remove bacteria followed by real-time PCR for indicated antagonists at 24h (*p<0.05; n = 3 independent experiments). **D. Effect of wild type CR infection on expression of EZH2 and WIF1 in YAMC cells**. Young Adult Mouse Colon (YAMC) cells were either uninfected (C) or infected with wild CR for 3h followed by measurement of EZH2 (**Di**) and WIF1 (**Dii**) expression levels at 24 and 72 h via real-time PCR (*p<0.05; n = 3 independent experiments). **E.** YAMC cells were also infected with either wild type CR or ΔescV mutant for 3h followed by measurement of EZH2 (**Ei**) and WIF1 (**Eii**) levels as described above (*p<0.05; n = 3 independent experiments). **F.** Effect of overexpressing EZH2 (**Fi**) on WIF1 (**Fii**) mRNA levels in YAMC cells. Two positive clones (EZH2-4 and EZH2-5) were selected (*p<0.05; n = 3 independent experiments).

Supplementary Figure 4. Reciprocal relationship between EZH2 and WIF1. A. Paraffin embedded sections from four mouse distal colons were co-stained with antibodies for EZH2 and WIF1. DAPI was used to label the nuclei. Representative photomicrographs showing merged images. Bar = 100μm (n = 2 independent experiments). Please note that areas positive for EZH2 are predominantly negative for WIF1. **B.** Paraffin embedded sections prepared from uninfected or CR infected *Apc*$^{Min/+}$ mice were also stained for EZH2 and WIF1. Bar = 100μm (n = 2 independent experiments).

Supplementary Figure 5. Mouse WIF1 promoter. The sequence was obtained from the mouse genome sequence database at the UCSF web server (http://genome.ucsf.edu) using the promoter search program (http://gpminer.mbc.nctu.edu.tw). A 1.66 kb fragment was amplified using mouse genomic DNA as a template. A standard PCR was used to amplify the regions. Sequences corresponding to the primers used

for the amplification are shown in boxes. Putative transcription start site (TSS) is marked with arrow. ATG open reading frame codon side (defined as +1) as well as the TATA box-containing region is in bold and underlined. E-box sequence is in boxes. Putative Stat3 (TTTCCC), NFAT (GGAAA), GATA (GATTA, GTTAT), Myc (CACGTG) and SP1 (CCGCC) binding sites are in bold. The underlined sequences in bold are the positions of WIF1-primers (WIF1-4 in Fig. 3A) used for the ChIP Assay.

Supplementary Figure 6. Both EZH2 and HDAC regulate WIF1 transcription. A. HEK-293T cells were transfected with human WIF1-Luc reporter vector for 24 hours followed by either depletion of EZH2 with specific siRNAs (**A**) or overexpression of EZH2 (**B**) followed by measurement of reporter activity ($^{\star}p<0.05$; n = 5 independent experiments). **C.** HEK-293T cells were treated with DZNep and SAHA either alone or in combination for 24 hours followed by measurement of reporter activity ($^{\star}p<0.05$; n = 5 independent experiments). **D.** Real-time PCR for EZH2 in uninfected (control) or CR infected YAMC cells treated with NaB (* and ** $p<0.05$; n = 3 independent experiments). **E.** Western blot for EZH2 and H3K27me3 in uninfected or CR infected YAMC cells treated with indicated amount of NaB. Actin was used as loading control. **F.** Real-time PCR for WIF1 in untreated (Cont.) or NaB-treated YAMC cells (*$p<0.05$; n = 3 independent experiments). **G-H.** WIF1 promoter activities in HCT116 colon cancer cells treated as described in **A** and **C** ($^{\star}p<0.05$; n = 5 independent experiments).