(shRNA) sequence against KLF5 or control shRNA. The lysates were collected from stable T84 knockdown or control cell lines and immunoblots performed for KLF5, Sox9 and actin (loading control) (Panel A). Lane 1 represents lysates from control shRNA transduction and lane 2 from KLF5-specific shRNA transduction. In Panel B, HCT116 cells were either mock-transfected (Lane 1), transfected with empty vector (Lane 2) or with pCI-Neo-KLF5 (Lane 3). Sox9 expression is inversely correlated to KLF5 expression in both T84 and HCT116 experiments (Panels A and B).

Figure 9 – Chromatin Immunoprecipitation (ChIP) assay show strong binding of KLF5 to Sox9, Reg1A and Cyclin D1 promoters.

Chromatin immunoprecipitation (ChIP) assays were performed as per Methods. HCT116 cells were transfected with KLF5, Sox9 or empty vector before collecting lysates for immunoprecipitations (IP) with Sox9, Reg1Aand cyclin D1 antibodies. DNA purified from the IP samples were then analysed with quantitative PCR using primers against Sox9, Reg1A and cyclin D1 respectively. Promoter binding affinity was plotted in the Y-Axis as Percent Input (%) against different promoter sequences in the X-axis. These results indicate that KLF5 strongly binds to Sox9, Reg1A and cyclin D1 promoters. Sox9 displays strong binding to Reg1A promoter and weak interactions with cyclin D1 and its own promoter.

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1 – Experimental schematic of tamoxifen treatment.

Eight week-old *Klf5*$^{ER\Delta}$ and control *Klf5*$^{fl/fl}$ mice were each injected IP with 1 mg tamoxifen dissolved in corn oil. Mice were injected for 5 consecutive days beginning on day 0 and sacrificed for tissue and RNA at days 3, 5, 7 and 11.

Supplementary Figure 2 – No significant change in weights of mice following tamoxifen treatment.

Klf5$^{ER\Delta}$ and control *Klf5*$^{fl/fl}$ mice were each injected IP with 1 mg tamoxifen dissolved in corn oil and weighted on 0, 4, 6 and 8 days post treatment. The results were plotted on a graph with weights on the Y-axis and days on the X-axis. The mice did not show any significant differences in weights between the two genotypes.

Supplementary Figure 3– Colonic crypts display acute inflammatory changes upon *Klf5* deletion.

Representative H&E images display colonic crypts of day 5 tamoxifen-treated *Klf5*$^{fl/fl}$ controls (Panel A) and days 3, 5, 7 & 11 after the start of tamoxifen treatment in *Klf5*$^{ER\Delta}$ mice (Panels B, C, D and E, respectively). Acute focal inflammation with reactive atypia, mild nuclear enlargement, prominent nucleoli and loss of goblet was evident in days 3 and 5 (Panels B & C) compared to the control (Panel A). Day 7 & 11 panels (D & E) show a moderate resolution of inflammation and restoration of goblet cells.

Supplementary Figure 4 – Ki-67 expression is reduced in the colons of *Klf5*$^{ER\Delta}$ and control mice following tamoxifen treatment.

Immunohistochemical analysis was performed on *Klf5*$^{ER\Delta}$ and control *Klf5*$^{fl/fl}$ mice. Brown color represents Ki-67 stain. Sections were counter-stained with Hematoxylin to stain nuclei blue. In the day 5 tamoxifen-treated control *Klf5*$^{fl/fl}$ mice, a strong Ki-67 staining pattern was located at the bottom of the colonic crypts (Panel A). Panels B, C, D and E represent *Klf5*$^{ER\Delta}$ mouse colons stained with Ki-67. Panels B and C show a decrease in Ki-67 staining at days 3 and 5 after the start of tamoxifen treatment, respectively. Ki-67 expression gradually returns to baseline by days 7 and 11 (Panels D and E, respectively).

Supplementary Figure 5 – Decrease in proliferation is observed immediately following *Klf5* deletion.

Eight week-old $Klf5^{ER\Delta}$ and control $Klf5^{fl/fl}$ mice were injected IP with 1 mg tamoxifen dissolved in corn oil for 5 consecutive days. Each day after tamoxifen injection, mice were also injected with bromodeoxyuridine (BrdU) for 4 hr before sacrificing and collecting intestinal tissues. Results were plotted with number of BrdU positive cells/crypt on the Y-axis and the days with tamoxifen treatment on the X-axis (Panel A). Staining for Ki-67 (Fig. 3) was quantified by counting the number of Ki-67 positive cells in 50 crypts per time point. These results were then plotted with number of Ki-67 positive cells/crypt on the Y-axis and days with tamoxifen treatment on the X-axis (Panel B). The number of BrdU and Ki-67 cells per crypt were reduced upon days of tamoxifen treatment compared with day 0 control (Panels A and B).

Supplementary Figure 6 – Zone of Sox9 expression is expanded in mouse colons following induced deletion of *Klf5*.

Colonic epithelial sections stained for Sox9 are represented in Panels A-E. Panel A shows control $Klf5^{fl/fl}$ staining. Panels B-D show day 3, 5, 7 and 11 tamoxifen-treated $Klf5^{ER\Delta}$ colon sections. Red brackets (Panels A, D and E) and red arrows (Panels B and C) mark epithelial cells staining positive for Sox9. Expansion of Sox9-positive zone was observed in Panels B-D. Restoration of Sox9 expansion to the bottom of crypts was observed in Panel E similar to control (Panel A).

Supplementary Figure 7 – Sox9 expression zone in $Klf5^{ER\Delta}$ crypts is expanded but not the staining intensity per cell compared to control

Cells staining positive for Sox9 (red stain from Fig. 7) were counted in each crypt for 3 sections and tallied against nuclear stain (DAPI, blue stain from Fig. 7) to obtain the

proportion of Sox9-positive cells per crypt. This data is represented as a percentage of total cells per crypt. Days 3, 5 and 7 tamoxifen treated mice show significantly increased numbers of Sox9-positive cells per crypt when compared to the control *Klf5*$^{fl/fl}$ mice. Positive staining and cell counts for Sox9 in both control and tamoxifen-treated samples were measured using ImageJ software (http://imagej.nih.gov/). ***- P<0.001, **- P<0.01

Supplementary Figure 8 – Cleaved caspase 3, chromogranin A and carbonic anhydrase staining show no significant changes in *Klf5*$^{ER\Delta}$ and control *Klf5*$^{fl/fl}$ mice.

Day 5 tamoxifen-treated *Klf5*$^{ER\Delta}$ and control *Klf5*$^{fl/fl}$ mice were stained with cleaved caspase 3 (Panels A & B), chromogranin A (Panels C & D) or carbonic anhydrase (Panels E & F) antibodies as described in Methods. Panels A & B show representative images of cleaved caspase 3 staining, denoting apoptosis, in control *Klf5*$^{fl/fl}$ and *Klf5*$^{ER\Delta}$ colon, respectively. Panels C & D show chromogranin A staining representing enteroendocrine cells in *Klf5*$^{fl/fl}$ and *Klf5*$^{ER\Delta}$ colonic crypts, respectively. Panels E & F show staining in *Klf5*$^{fl/fl}$ and *Klf5*$^{ER\Delta}$ colon for carbonic anhydrase representing cells of the absorptive lineage. There was no change in staining between the *Klf5*$^{fl/fl}$ and *Klf5*$^{ER\Delta}$ mice in each treatment condition.

Supplementary Figure 9 – KLF5 knock-down increases Sox9 promoter activity and its expression

DLD1 colon cancer cells were treated with *Klf5*-specific siRNA or control siRNA for 24 hours and then transfected with luciferase linked promoters for Sox9 and Reg1A. Relative luciferase reporter activity was measured and plotted on the Y-Axis against promoter transfection in Panel A. Panel B shows western blot analysis performed on cell lysates prior to measurement of luciferase reporter activity. Results show that Klf5 knock-down can significantly increase Sox9 promoter and expression but not Reg1A promoter activity.