**Supplementary Figure Legends**

**Supplementary Figure 1. Stable knockdown (KD) pools of STRAP were established by lentiviral vector and STRAP KD increases the sensitivity of colorectal cancer (CRC) cells to drug. A.** Expression of STRAP in stable pools from HCT116 and DLD-1 parental cells after transfection of STRAP shRNA (#1 and #2) was examined by western blotting. β-actin was used as a loading control. **B and C.** IC50 of stable STRAP KD cells compared with that of scrambled controls was determined by MTT assay after treating with the indicated drugs for 72 hrs. **D.** Representative Hematoxylin-Eosin (HE) stained images are shown. Magnification, X40. The expression of STRAP, Ki67 and cleaved caspase-3 in the tumor from DLD-1 clones (shCtrl and shSTRAP#1) was detected by IHC as described in Figure 1E. **E.** Expression of STRAP in stable pools from four parental cells after transfection of STRAP shRNA (#1and #2) was examined by western blotting. β-actin was used as a loading control.

**Supplementary Figure 2. Loss of STRAP diminishes the percentage of CD133+/CD44+** **subpopulation in CRC cell lines. A.** The fraction of CD133+/CD44+ cells in stable STRAP knockdown CRC cell lines (WiDR, LoVo, HCT116, HT29, RKO and DLD-1) was analyzed by flow cytometry. The percentages of double positive subpopulation are shown in Q2 area. **B.** HE staining for the overall tumor morphology was conducted using tissues from AOM/DSS mouse model (Figure F-I). Pictures are representative of both normal tissue and tumor segments from Strap+/+ and Strap+/- mice.

**Supplementary Figure 3. STRAP regulates the expressions of CSC markers. A.** The mRNA levels of CD133, CD44, NANOG, SLUG, SNAIL, BMI1, and SOX2 in STRAP knockdown cells (HCT116, DLD-1 and SW620) as well as in control cells were determined by qPCR and shown as the relative fold changes using GAPDH as a loading control. Each experiment was performed at least thrice with similar results. \**P*< 0.05, shSTRAP#1 vs. shCtrl. **B.** Protein expressions of NOTCH components in shSTRAP#2 and shCtrl clones from HCT116 and DLD-1 cell lines were analyzed by western blot analyses using antibodies against the corresponding proteins as indicated.

**Supplementary Figure 4. Reduced expression of STRAP leads to the distribution of H3K27me3 on the loci of NOTCH signaling genes**. Anti-H3K4me3 and -H3K27me3 antibodies were used for ChIP assays using shSTRAP#1 and shCtrl clones from HCT116 and DLD-1 cell lines. qPCR amplification was done to verify the data obtained from ChIP panel assays (Figure 4A). n=3, \**P*< 0.05, \*\**P*< 0.01, compared to control.

**Supplementary Figure 5. STRAP does not bind with EZH2 or EED and does not regulate their expression**. **A.** Total cell lysates from shSTRAP#2 and shCtrl clones were analyzed for H3K27me3, EZH2, SUZ12, and β-actin expression by immunoblotting with their respective antibodies. **B and C.** 293T cells were co-transfected with HA-tagged EZH2 or EED and Flag-tagged STRAP as indicated. Lysates were subjected to reciprocal immunoprecipitation with either anti-HA or anti-Flag antibody and the co-precipitated proteins were analyzed by western blotting. Protein expressions were also tested by western blotting the lysates.

**Supplementary Figure 6. Depletion of STRAP reduces the transcriptional levels of NOTCH signaling effectors and CSC markers in sphere-derived cells**. HCT116 and DLD-1 cells were incubated in stem-cell medium for 5 days to form spheres followed by infection with lentivirus containing shSTRAP#1 or shCtrl vector. The expression of STRAP was tested by western blotting using anti-STRAP antibody (Figure 5C). Total RNAs were extracted from the above cells and qPCR assays were performed using primers targeting several CSC-and NOTCH-related genes. n=3, \**P*< 0.05, \*\**P*< 0.01, compared to control.