Supplemental Figure Legend

Figure S1. Sensitivity of colon carcinoma cell lines to FasL-induced apoptosis in vitro. A. Murine (CT26, MC32a and MC38) colon carcinoma cells were cultured in the presence of FasL at the indicated doses for 24h. Human (HCT116 and SW480) colon carcinoma cells were cultured in the presence of FasL for 72h. Both floating and adherent cells were collected, stained with Annexin V and PI, and analyzed by flow cytometry. **B**. Apoptosis ($Annexin^+PI^-$) and apoptotic cell death ($Annexin\ V^+PI^+$) were quantified for each cell lines. Shown are representative dot plots of one of two independent experiments.

Figure S2. Loss of Fas function leads to resistance to FasL-induced apoptosis. A. WT (MC78) and Fas-deficient Fas^{lpr} (MC69) mice were injected with MCA to induce spontaneous sarcoma. The tumors were excised from the tumor-bearing mice, digested with collagenase and cultured to establish stable Fas WT and Fas-deficient sarcoma cells lines. Cell blocks were prepared, sectioned and stained with H&E. Shown are of the cellular neoplasm of spindled cells with pleomorphism and high mitotic activity of one pair of Fas WT (MC78) and Fas-deficient (MC69) cell lines. Top panel: low magnification; bottom panel: high magnification. **B.** Fas WT (MC78 and MC693) and Fas-deficient (MC68 and MC69) sarcoma cell lines were treated FasL at the indicated concentrations for 72h. Both floating and adherent cells were collected, stained with Annexin V and PI, and analyzed by flow cytometry. C. Apoptosis ($Annexin^+PI^-$) and apoptotic cell death ($Annexin\ V^+PI^+$) were quantified for each cell lines. Shown are representative dot plots of one of two independent experiments. **D**. The sarcoma cell lines were treated with FasL as in B and C for 72h. ^{3}H-thymidine were added to the cells and cultured for

5h. 3H incorporation was quantified using a liquid scintillation spectrophotometer. Data are representatives of three independent experiments.

Figure S3. FasL induces apoptosis to increase the frequency of colon cancer stem cell-like cells in MSS colon cancer cells. A. MSI (HCT11) and MSS (SW480) human colon carcinoma cell lines were cultured with IFNγ (100 U/ml) and TNFα (100 U/ml) in the absence or presence of FasL (50 ng/ml), Z-VAD (20 μM) or both FasL and Z-VAD for 24h. Cells were then stained with CD133-, CD24-specific mAbs and DAPI, and analyzed by flow cytometry. $CD133^{+}CD24^{lo}$ cells were quantified. Shown are representative flow cytometry dot plots gated on live singllet cells. **B**. $CD133^{+}CD24^{lo}$ cells as shown in A were quantified. Column: mean; Bar: SD. Data are representative result of one of two independent experiments.

Figure S4. $CD133^{+}CD24^{lo}$Faslo colon cancer stem cell-like cells exhibit increased invasiveness in vitro. **A**. $CD133^{+}CD24^{lo}Fas^{lo}$ and $CD133^{+}CD24^{hi}Fas^{hi}$ CT26 cells were cultured in the upper chamber of the Falcon insert in a 24 well plate for 24h. Cells that had migrated to the underside of the falcon insert were fixed in 4% PFA, stained with Crystal Violet. Representative images of stained cells of one of three independent experiments are shown at the left panel. The migrated cells were quantified and presented at the right panel. **B**. $CD133^{+}CD24^{lo}Fas^{lo}$ and $CD133^{+}CD24^{hi}Fas^{hi}$ CT26 cells were cultured to confluent. The cell monolayer was scratched with a pipet tip. Shown are representative images of the scratch areas 24h after scratch of one of three independent experiments (top panel). Scratch closure rate was calculated initial scratch width (μm) - final scratch width (μm)/time.

Figure S5. Overexpression of Fas does not alter sphere formation. **A**. SW480-FasLR and SW480-FasLR-Fas cells were stained with Fas-specific mAb and analyzed by flow cytometry. Shown is Fas MFI. **B**. The tumor cells were cultured in the presence of FasL at the indicated concentrations for 24h. Cells were stained with Annexin V and PI and analyzed by flow cytometry for apoptotic cell death. **C**. Cells were cultured in ultra-low attachment plate as described in the method section for 10 days and quantified for sphere formation.