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**Fig. S3. TIMP3 and MMP3 immunofluorescence in hepatic metastases and analysis of MMP3 knockdown and TIMP3 overexpression.** (A-C) Immunofluorescence analysis of the effects of in vivo treatment with Senexin B (33 mg/kg, p.o., daily, in combination with Senexin B-containing diet) or vehicle control on days 11-14 after splenic inoculation on the expression of TIMP3 and MMP3 proteins in hepatic metastases. A: TIMP3 staining in the tumor areas. B: TIMP3 staining in the tumor-free liver areas. C: MMP3 staining in the tumor areas. Scale bars: 20 μm. (D) Analysis of the overexpression of human TIMP3 in CT26-VTIMP3 and control CT26-VGFP cells (qPCR, in duplicates). (E) Analysis of CDK8 and mouse TIMP3 expression in VTIMP3 and VGFP cells (qPCR, in duplicates). (F) Dynamics of cell growth of VTIMP3 and VGFP cells. (G) Dynamics of VTIMP3 and VGFP tumor growth s.c. (H) Images of representative orthotopic tumors formed by VTIMP3 and VGFP cells after cecal injection. (I) Analysis of MMP3 knockdown by five different shRNAs. (J) qPCR analysis of CDK8 and TIMP3 expression in MMP3 knockdown cells (qPCR, in duplicates). (K) Effect of MMP3 knockdown on CT26 cell growth (n = 10) (L) Effect of MMP3 knockdown on the dynamics of CT26 tumor growth s.c. (n = 10).

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**Fig.S4. Supplemental data for SMAD4, CTNNB1 and STAT1 knockdown and microRNA analysis.** (A) Effects of SMAD4 (left) and CTNNB1 (right) knockdown of on CT26 cell proliferation; cell numbers measured in duplicates (B) Effects of SMAD4 knockdown on the expression of MMP3 in the absence or presence of 1 μM Senexin B (24 hr treatment) (qPCR, in duplicates). (C) Effects of CTNNB1 knockdown on the expression of TIMP3 in the absence or presence of 1 μM Senexin B (24 hr treatment) (qPCR, in duplicates). (D) Expression of the indicated microRNAs in CT26-pLKO.1, CT26-shCDK8 or CT26-shSMAD4 cells untreated or treated with 1 µM Senexin B (qPCR, in duplicates). (E) CDK8 expression in the indicated CT26 derivatives transfected with miR-control mimic, miR-181b mimic or miR-181b inhibitor in the presence of 1 µM Senexin B (qPCR, in duplicates). \* denotes P<0.05, \*\* denotes P<0.01 and \*\*\* denotes P<0.001. (F) STAT1 knockdown by two different shRNAs in CT26 cells (immunoblotting). (G) Expression of MMP3 and TIMP3 in CT26 cells with STAT1 knockdown (qPCR, in duplicates).

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**Fig.S5. Effects of Senexin B on the expression of different MMPs in a panel of human colon cancer cell lines and human colon cancer growth s.c.** (A) Effects of Senexin B on the expression of different MMPs in a panel of human colon cancer cell lines. qPCR analysis of the effects of 1 µM Senexin B (24 h treatment) on the expression of the indicated MMPs in the indicated cell lines (qPCR, in duplicates). (B) Dynamics of HCT116 tumor growth s.c. in mice treated with Senexin B (33 mg/kg, p.o., b.i.d) or vehicle control (n = 5).