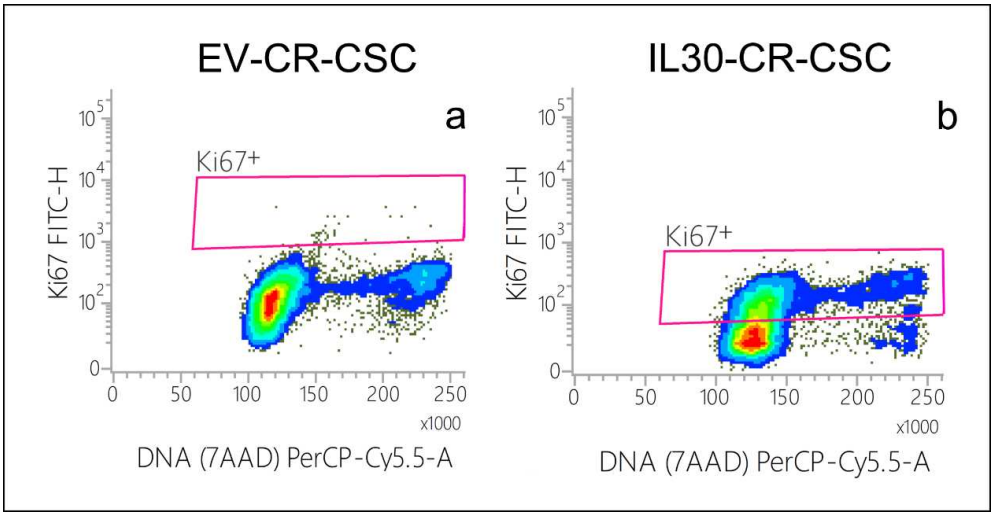
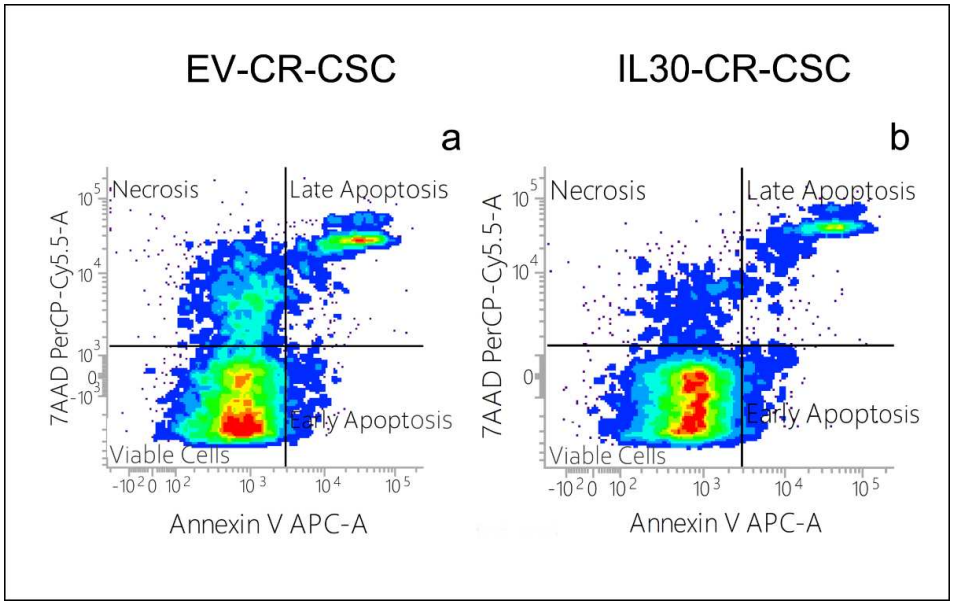
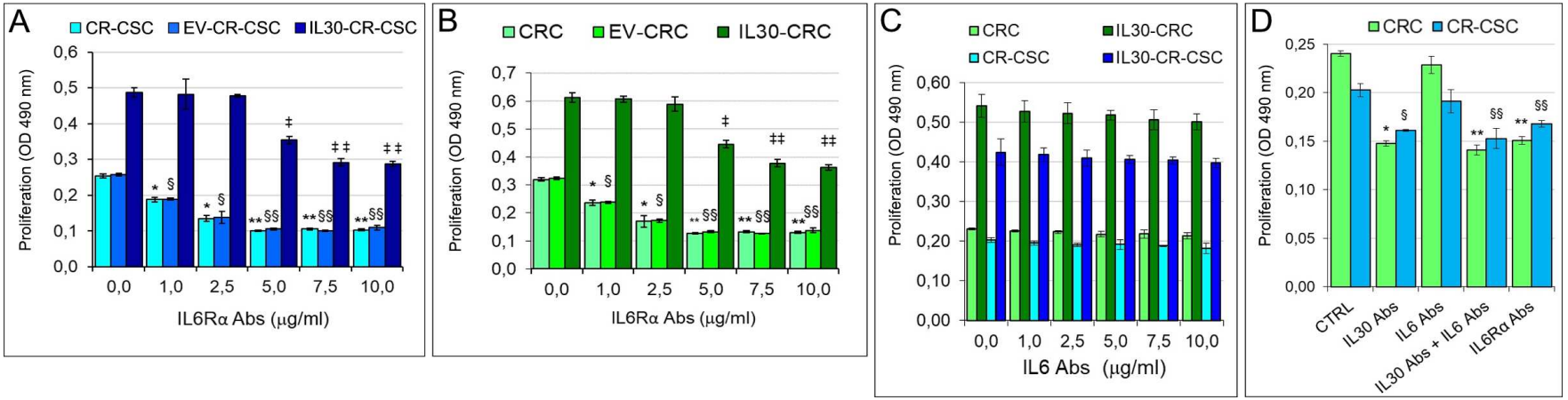


Supplemental Figure S1. Flow cytometric analysis of cell proliferation by Ki67 staining. The areas inside the purple lines show Ki67$^+$proliferating cells. The percentage of Ki67$^+$ proliferating cells (calculated by comparison with the unstained cells) was 0.5% for EV-transfected CR-CSCs (a) and 46% for IL30-overexpressing CR-CSCs (b). Results from WT CR-CSCs are comparable with those from EV-transfected cells. Red zones: high density of events; blue zones: low density of events. Experiments were performed in triplicate. 7-AAD: 7-aminoactinomycin D. These results were comparable to those obtained by Ki67 staining in IL30-CRC *versus* control cells.



Supplemental Figure S2. Flow cytometric analysis of cell apoptosis by annexin V assay. The four quadrants of each plot depict the following: lower left = Viable Cells (Annexin V–, 7-AAD–), lower right = Early Apoptotic Cells (Annexin V+, 7-AAD–), upper right = Late Apoptotic Cells (Annexin V+, 7-AAD+), upper left = Necrotic Cells (Annexin V–, 7-AAD+). There was no difference in the percentage of apoptotic events between EV-transfected (a) and IL30-overexpressing cells (b). Results from WT CR-CSCs are comparable with those from EV-transfected cells. Red zones: high density of events; blue zones: low density of events. 7-AAD: 7-aminoactinomycin D. These results were comparable to those obtained by annexin V assay in IL30-CRC *versus* control cells. Experiments were performed in triplicate.



Supplemental Figure S3

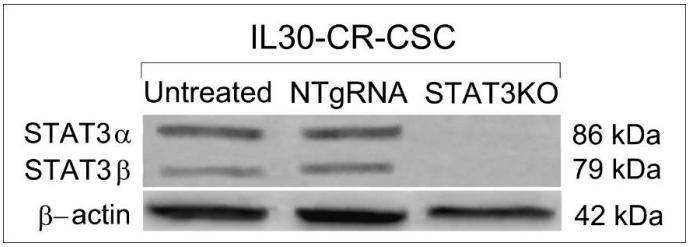
A. MTT-assay of CR-CSCs, EV-CR-CSCs and IL30-CR-CSCs, treated or not with anti-IL6Rα Abs for 48h. ANOVA: $p<0.0001$. $^{*,\S}p<0.01$, Tukey HSD Test compared with CR-CSCs or EV-CR-CSCs treated with 0.0 μg/ml. $^{**,\S\S,\ddagger}p<0.01$, Tukey HSD Test compared with CR-CSCs, EV-CR-CSCs or IL30-CR-CSCs treated with 0.0, 1.0 or 2.5 μg/ml. $^{\ddagger\ddagger}p<0.05$, Tukey HSD Test compared with IL30-CR-CSCs treated with 0.0, 1.0, 2.5 or 5.0 μg/ml. At each concentration of anti-IL6Rα Abs, the proliferation of IL30-CR-CSCs was significantly higher compared to the proliferation of both CR-CSCs and EV-CR-CSCs (ANOVA: $p<0.0001$). The results were comparable to those obtained from CRC cells, EV-CRC and IL30-CRC cells.

B. MTT assay of CRC, EV-CRC and IL30-CRC cells, treated or not with anti-IL6Rα Abs for 48h. ANOVA: $p<0.0001$. $^{*,\S}p<0.01$, Tukey HSD Test compared with CRC or EV-CRC cells treated with 0.0 μg/ml. $^{**,\S\S,\ddagger}p<0.01$, Tukey HSD Test compared with CRC, EV-CRC

or IL30-CRC cells treated with 0.0, 1.0 or 2.5 µg/ml. ‡‡$p<0.05$, Tukey HSD Test compared with IL30-CRC cells treated with 0.0, 1.0, 2.5 or 5.0 µg/ml. At each concentration of anti-IL6Rα Abs, the proliferation of IL30-CRC cells is significantly higher compared to the proliferation of both CRC and EV-CRC cells (ANOVA: $p<0.0001$; Tukey HSD Test: $p<0.01$).

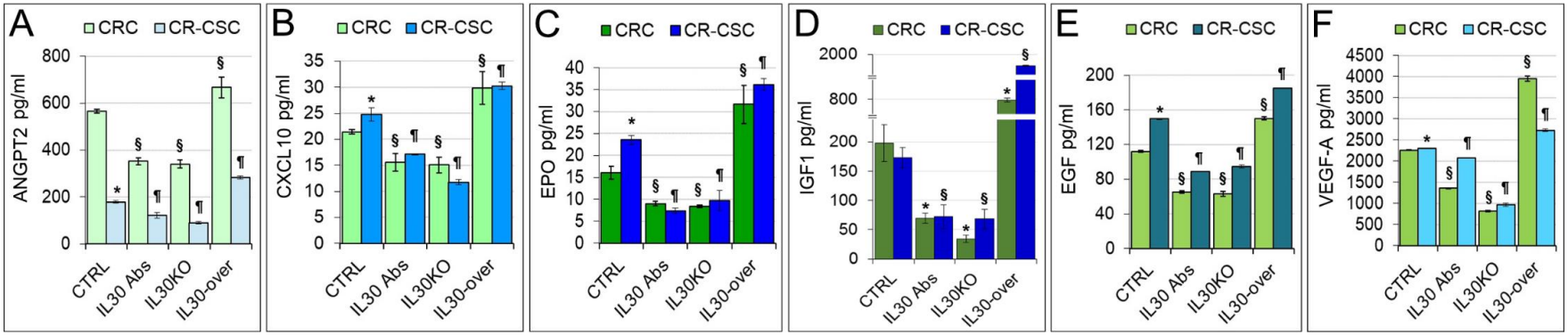
C. MTT-assay of CRC cells, IL30-CRC cells, CR-CSCs and IL30-CR-CSCs treated with anti-IL6-Abs. ANOVA: $p>0.05$ (not significant).

D. MTT-assay of CRC cells and CR-CSCs untreated (CTRL) or treated with anti-IL30 Abs, anti-IL6 Abs, anti-IL30 + anti-IL6 Abs and anti-IL6Rα Abs (all antibodies used at 5 µg/mL). ANOVA: $p<0.0001$. *,§$p<0.01$, Tukey HSD Test compared with untreated CRCs or CR-CSCs. **,§§$p<0.05$, Tukey HSD Test compared with CRCs or CR-CSCs untreated or treated with anti-IL6 Abs.



Supplemental Figure S4.

Western Blot analysis of STAT3α and β protein expression in untreated IL30-CR-CSCs, NTgRNA-treated IL30-CR-CSCs and STAT3 gene-deleted IL30-CR-CSCs.



Supplemental Figure S5.

A. ELISA-assay of ANGPT2 release by WT CRC cells and CR-CSCs, untreated (CTRL), treated with anti-IL30 Abs (15 µg/ml or 10 µg/ml, respectively), IL30-gene transfected (IL30-over) or deleted (IL30KO). *Student's *t*-test: $p<0.0001$, compared with untreated CRC cells. ANOVA: $p<0.0001$. §$p<0.01$, Tukey HSD Test compared with untreated CRC cells. ¶$p<0.01$, Tukey HSD Test compared with untreated CR-CSCs. Results obtained from NTgRNA-treated and EV-transfected cells were comparable with those from WT cells. Experiments were performed in triplicate.

B. ELISA-assay of CXCL10 release by WT CRC cells and CR-CSCs, untreated (CTRL), treated with anti-IL30 Abs (15 µg/ml or 10 µg/ml, respectively), IL30 gene-transfected (IL30-over) or deleted (IL30KO). *Student's *t*-test: $p=0.035$, compared with untreated CRC cells. ANOVA: $p<0.01$. §$p<0.05$, Tukey HSD Test compared with untreated CRC cells. ¶$p<0.01$, Tukey HSD Test compared with untreated CR-CSCs. Results obtained from NTgRNA-treated and EV-transfected cells were comparable with those from WT cells. Experiments were performed in triplicate.

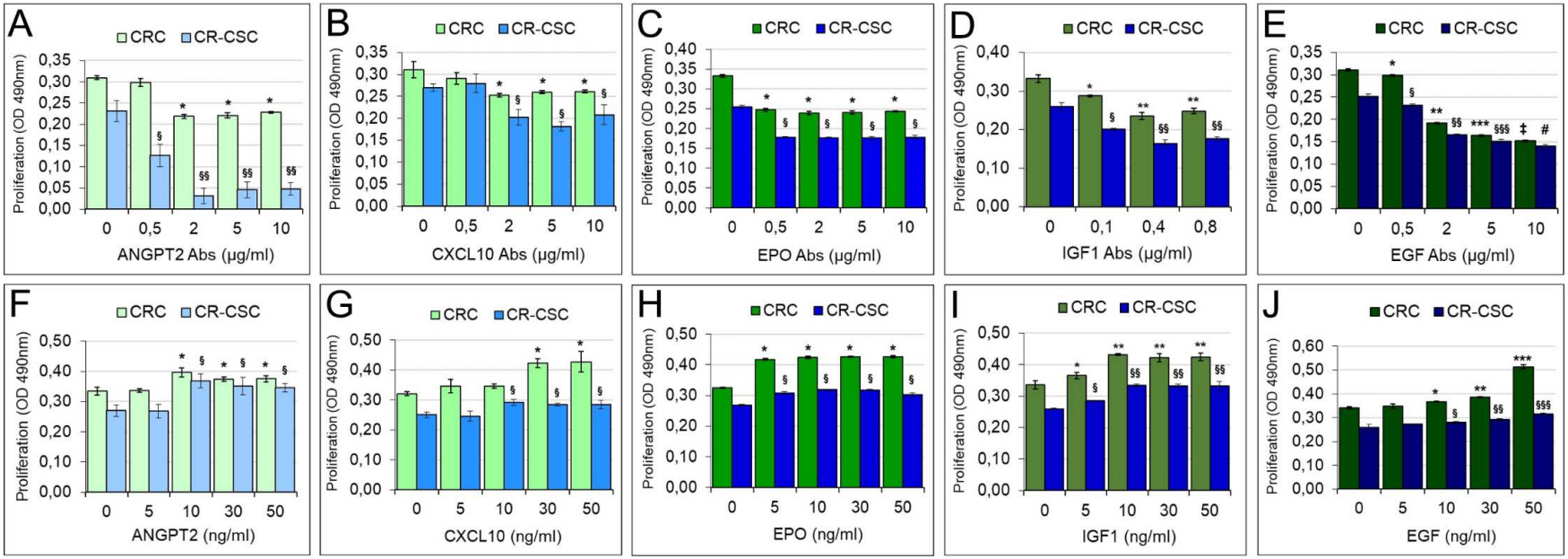
C. ELISA-assay of EPO release by CRC cells and CR-CSCs untreated (CTRL), treated with anti-IL30 Abs (10 µg/ml), IL30 gene-transfected (IL30-over) or deleted (IL30KO). *Student's *t*-test: p=0.003, compared with untreated CRC cells. ANOVA: p<0.0001. §p<0.05, Tukey HSD Test compared with untreated CRC cells. ¶p<0.01, Tukey HSD Test compared with untreated CR-CSCs. Results obtained from NTgRNA-treated and EV-transfected cells were comparable with those from WT cells. Experiments were performed in triplicate.

D. ELISA-assay of IGF1 release by CRC cells and CR-CSCs untreated (CTRL), treated with anti-IL30 Abs (10 µg/ml), IL30 gene-transfected (IL30-over) or deleted (IL30KO). ANOVA: p<0.0001. *p<0.01, Tukey HSD Test compared with untreated CRC cells. §p<0.01, Tukey HSD Test compared with untreated CR-CSCs. Results obtained from NTgRNA-treated and EV-transfected cells were comparable with those from WT cells. Experiments were performed in triplicate.

E. ELISA-assay of EGF release by CRC cells and CR-CSCs untreated (CTRL), treated with anti-IL30 Abs (10 µg/ml), IL30 gene-transfected (IL30-over) or deleted (IL30KO). *Student's *t*-test: p<0.0001, compared with untreated CRC cells. ANOVA: p<0.0001. §p<0.01, Tukey HSD Test compared with untreated CRC cells. ¶p<0.01, Tukey HSD Test compared with untreated CR-CSCs. Results obtained from NTgRNA-treated and EV-transfected cells were comparable with those from WT cells. Experiments were performed in triplicate.

F. ELISA-assay of VEGF-A release by WT CRC cells and CR-CSCs, untreated (CTRL), treated with anti-IL30 Abs (10 µg/ml), IL30 gene-transfected (IL30) or deleted (IL30KO). *Student's *t*-test: p<0.001, compared with untreated CRC cells. ANOVA: p<0.0001. §p<0.01, Tukey HSD Test compared with untreated CRC cells. ¶p<0.01, Tukey HSD Test compared with untreated CR-CSCs. Results obtained from NTgRNA- and EV-

transfected cells were comparable with those from WT cells. Experiments were performed in triplicate.



Supplemental Figure S6.

A. MTT assay of CRC cells and CR-CSCs untreated (0 µg/ml) or treated with anti-ANGPT2 Abs. ANOVA: $p<0.0001$. *$p<0.01$, Tukey HSD Test compared with CRC cells untreated and treated with 0.5 µg/ml. §$p<0.01$, Tukey HSD Test compared with untreated CR-CSCs. §§$p<0.05$, Tukey HSD Test compared with CR-CSCs untreated and treated with 0.5 µg/ml. Experiments were performed in triplicate.

B. MTT assay of CRC cells and CR-CSCs untreated (0 µg/ml) or treated with anti-CXCL10 Abs. ANOVA: $p<0.001$. *$p<0.05$, Tukey HSD Test compared with CRC cells untreated and treated with 0.5 µg/ml. §$p<0.01$, Tukey HSD Test compared with CR-CSCs untreated and treated with 0.5 µg/ml. Experiments were performed in triplicate.

C. MTT assay of CRC cells and CR-CSCs untreated (0 µg/ml) or treated with anti-EPO Abs. ANOVA: $p<0.0001$. *$p<0.01$, Tukey HSD Test compared with untreated CRC cells. §$p<0.01$, Tukey HSD Test compared with untreated CR-CSCs. Experiments were performed in triplicate.

D. MTT assay of CRC cells and CR-CSCs untreated (0 µg/ml) or treated with anti-IGF1 Abs. ANOVA: $p<0.0001$. *$p<0.01$, Tukey HSD Test compared with untreated CRC cells. **$p<0.01$, Tukey HSD Test compared with CRC cells untreated and treated with 0.1 µg/ml. §$p<0.01$, Tukey HSD Test compared with untreated CR-CSCs. §§$p<0.01$, Tukey HSD Test compared with CR-CSCs untreated and treated with 0.1 µg/ml. Experiments were performed in triplicate.

E. MTT assay of CRC cells and CR-CSCs untreated (0 µg/ml) or treated with anti-EGF Abs. ANOVA: $p<0.0001$. *$p<0.01$, Tukey HSD Test compared with untreated CRC cells. **$p<0.01$, Tukey HSD Test compared with CRC cells untreated and treated with 0.5 µg/ml. ***$p<0.01$, Tukey HSD Test compared with CRC cells untreated and treated with 0.5 and 2 µg/ml. ‡$p<0.01$, Tukey HSD Test compared with CRC cells untreated and treated with 0.5, 2 and 5 µg/ml. §$p<0.01$, Tukey HSD Test compared with untreated CR-CSCs. §§$p<0.01$, Tukey HSD Test compared with CR-CSCs untreated and treated with 0.5 µg/ml. §§§$p<0.05$, Tukey HSD Test compared with CR-CSCs untreated and treated with 0.5 and 2 µg/ml. #$p<0.01$, Tukey HSD Test compared with CR-CSCs untreated and treated with 0.5, 2 and 5 µg/ml. Experiments were performed in triplicate.

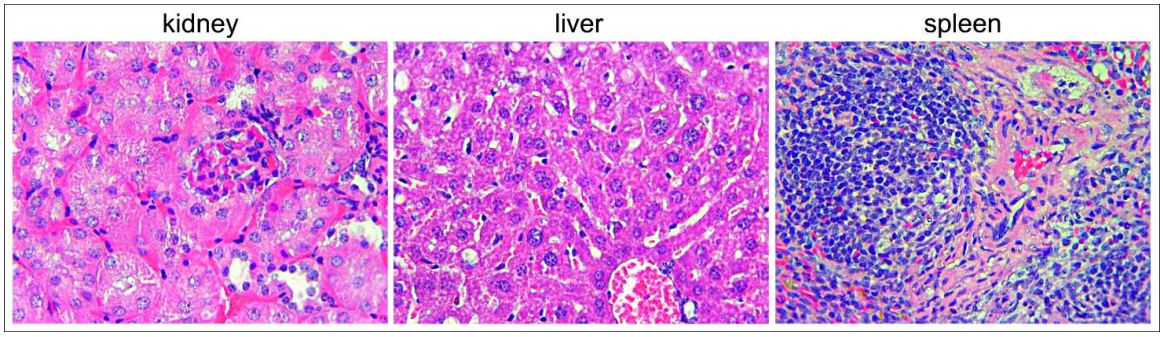
F. MTT assay of CRC cells and CR-CSCs untreated (0 ng/ml) or treated with recombinant ANGPT2. ANOVA: $p<0.001$. *$p<0.05$, Tukey HSD Test compared with CRC cells untreated and treated with 5 ng/ml. §$p<0.05$, Tukey HSD Test compared with CR-CSCs untreated and treated with 5 ng/ml. Experiments were performed in triplicate.

G. MTT assay of CRC cells and CR-CSCs untreated (0 ng/ml) or treated with recombinant CXCL10. ANOVA: $p<0.01$. *$p<0.01$, Tukey HSD Test compared with CRC cells untreated and treated with 5 and 10 ng/ml. §$p<0.05$, Tukey HSD Test compared with CR-CSCs untreated and treated with 5 ng/ml. Experiments were performed in triplicate.

H. MTT assay of CRC cells and CR-CSCs untreated (0 ng/ml) or treated with recombinant EPO. ANOVA: $p<0.0001$. *$p<0.01$, Tukey HSD Test compared with untreated CRC cells. §$p<0.05$, Tukey HSD Test compared with untreated CR-CSCs. Experiments were performed in triplicate.

I. MTT assay of CRC cells and CR-CSCs untreated (0 ng/ml) or treated with recombinant IGF1. ANOVA: $p<0.0001$. *$p<0.05$, Tukey HSD Test compared with untreated CRC cells. **$p<0.01$, Tukey HSD Test compared with CRC cells untreated and treated with 5 ng/ml. §$p<0.05$, Tukey HSD Test compared with untreated CR-CSCs. §§$p<0.05$, Tukey HSD Test compared with CR-CSCs untreated and treated with 5 ng/ml. Experiments were performed in triplicate.

J. MTT assay of CRC cells and CR-CSCs untreated (0 ng/ml) or treated with recombinant EGF. ANOVA: $p<0.0001$. *$p<0.05$, Tukey HSD Test compared with CRC cells untreated and treated with 5 ng/ml. **$p<0.05$, Tukey HSD Test compared with CRC cells untreated and treated with 5 and 10 ng/ml. ***$p<0.01$, Tukey HSD Test compared with CRC cells untreated and treated with 5, 10 and 30 ng/ml. §$p<0.05$, Tukey HSD Test compared with untreated CR-CSCs. §§$p<0.05$, Tukey HSD Test compared with CR-CSCs untreated and treated with 5 ng/ml. §§§$p<0.01$, Tukey HSD Test compared with CR-CSCs untreated and treated with 5, 10 and 30 ng/ml. Experiments were performed in triplicate.



Supplemental Figure S7.

Histological aspects of kidney, liver and spleen of NSG mice bearing control tumors induced by the subcutaneous implantation of wild type CR-CSCs. At the end of the experiment, the organs were histologically normal and free from metastatic lesions, Magnification: X400.