Multimodal targeting of tumor vasculature and cancer stem-like cells in sarcomas with VEGF-A inhibition, HIF-1α inhibition, and hypoxia-activated chemotherapy

Supplementary Material
Supplemental Figure S1. (A) Graph of KP tumor size over time following treatment with DC101 or control IgG. (B) Graphs and photos of tumors stained for CD31 immunohistochemistry. (C) Graphs and photos of tumors stained for Hypoxyprobe. Bars represent standard deviation.

Supplemental Figure S2. Graphs of total apoptosis based on cleaved caspase 3 (CCR3) expression, endothelial cell (EC)-specific apoptosis based on CD31 and CCR3 co-expression, and non-EC-specific apoptosis based on CCR3 expression in CD31-negative cells. Bars represent standard deviation. **p<0.05 compared to all other groups.
Supplemental Figure S3. HT1080 xenografts treated with multimodal therapy. Photos (A) and graphs (B) of PCNA immunohistochemistry for proliferation, TUNEL immunofluorescence for total apoptosis, CD31 and TUNEL immunofluorescence for endothelial cell (EC)-specific apoptosis, CD31 immunohistochemistry for microvessel density, and nuclear HIF-1α.
immunohistochemistry for HIF-1α activity. Bars represent standard deviation. **p<0.05 compared to all other groups.

Supplemental Figure S4. SK-LMS-1 xenografts treated with multimodal therapy. Photos (A) and graphs (B) of PCNA immunohistochemistry for proliferation, TUNEL immunofluorescence for total apoptosis, CD31 and TUNEL immunofluorescence for endothelial cell (EC)-specific...
apoptosis, CD31 immunohistochemistry for microvessel density, and nuclear HIF-1α immunohistochemistry for HIF-1α activity. Bars represent standard deviation. **p<0.05 compared to all other groups.

Supplemental Figure S5. Multimodal therapy effects on proliferation, DNA damage, and apoptosis. Proliferation assay (A), γH2AX IF expression (B), and cleaved caspase 3 IF
expression (C) in HUVEC and SK-LMS-1 human leiomyosarcoma cells after VEGF withdrawal (No VEGF), evofosfamide (Evo, 10 μM), and/or low dose doxorubicin (Dox, 0.005 μM). All experiments done in normoxia and hypoxia. Bars represent standard deviation. *p<0.05 compared to control. **p<0.05 compared to all other groups.

|          | HT1080 | SK-LMS | MS4515 |
|----------|--------|--------|--------|
|          | Monlayers | Spheroids | Monlayers | Spheroids | Monlayers | Spheroids |
| CD271    | ![Image] | ![Image] | ![Image] | ![Image] | ![Image] | ![Image] |
| TNAP     | ![Image] | ![Image] | ![Image] | ![Image] | ![Image] | ![Image] |
| CD133    | ![Image] | ![Image] | ![Image] | ![Image] | ![Image] | ![Image] |
| CD44     | ![Image] | ![Image] | ![Image] | ![Image] | ![Image] | ![Image] |
| β-actin  | ![Image] | ![Image] | ![Image] | ![Image] | ![Image] | ![Image] |

**Supplemental Figure S6.** Western blot analysis of sarcoma cell lines grown as monolayers and as spheroids for putative sarcoma stem-like cell markers.