SUPPLEMENTAL DATA

Supplemental Materials & Methods

Dextran permeability studies

Mice were pretreated with chemotherapy and 4 days later, rhodamine B-conjugated dextran (70,000 MW, Invitrogen, Grand Island, NY) was dissolved in PBS and injected iv in a concentration of 100 mg/kg. 30 minutes later, mice were sacrificed and lungs were taken out for immediate CLSM visualization of the vasculature.

FACS analysis of Peripheral Blood

Blood was withdrawn by cardiac punction 4 days after treatment with cisplatin or vehicle control. Red blood cells were lysed and remaining blood cells were stained with fluorochrome-conjugated antibodies, followed by red cell lysis and acquisition by FACS.

Supplemental Figure Legends

Supplemental Figure 1. No vascular leakage is observed at the time of tumor cell injection.
Four days after cisplatin treatment, rhodamine-conjugated dextran was injected iv 30 minutes before sacrificing the mice. Vascular leakage was analyzed immediately after sacrifice by CLSM.

Supplemental Figure 2. C26-mCh cells are fluorescent and proliferate similarly to C26 parental cells.
An mCherry+ clone that **(A)** showed clear fluorescence *in vitro* and **(B)** comparable proliferation as determined by MTT analysis was selected for *in vivo* analysis. **(C)** After iv injection of tumor cells into the tail vein, the mCherry-expressing clone developed a similar amount of surface metastases after 2 weeks, compared to the parental C26 cell line. **(D)** Fluorescence was maintained 2 weeks after iv injection; ns: not significant.

Supplemental Figure 3. (A) For adhesion assays, bEND.3 EC monolayers that had been pretreated with cisplatin or vehicle control were stimulated for 4 hours with TNFα combined with PMA. Tumor cells were calcein-labeled and added to the wells. After 50 minutes, non-adherent tumor cells were removed and baseline fluorescence was measured. Then, wells were washed three times and after the third wash adhering C26 cells were determined by fluorescence analysis; The ratio between adherent cells after wash 3 versus adherent cells after initial tumor cell removal was calculated. **(B)** in

separate experiments, bEND.3 EC monolayers that had been pretreated with cisplatin or vehicle control were stimulated for 4 hours with TNFα. Tumor cells were calcein-labeled, incubated with antibodies targeting VCAM-1 or ICAM-1, and added to the EC monolayers. The ratio between adherent cells after wash 3 versus adherent cells after initial tumor cell removal was calculated. **(C)** bEND.3 EC monolayers that had been pretreated with cisplatin or vehicle control were stimulated for 4 hours with TNFα. C26 tumor cells were calcein-labeled and blocking antibodies to integrin β1 or β3 were added to the tumor cells. Subsequently, tumor cells were placed onto EC monolayers and adhesion experiments were performed. The ratio between adherent cells after wash 3 versus adherent cells after initial tumor cell removal was calculated. Cis: cisplatin, * $p<0.05$, ** $p<0.01$, ***$p<0.001$.

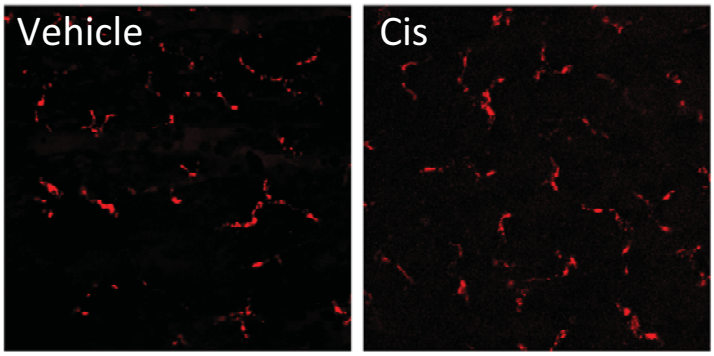
Supplemental Figure 4. Hematopoietic cells in lungs and circulation after cisplatin treatment.

(A,B,C) Mouse lungs were harvested four days after treatment with cisplatin or vehicle control. Single cell samples were prepared and stained for flow cytometry analysis of **(A)** VEGFR-1+CD11b+ myeloid cells, **(B)** VEGFR-1+CD45+CD117+ hematopoietic progenitor cells and **(C)** VEGFR-1+CD45+CXCR4+ hemangiocytes. **(D,E)** Blood was withdrawn four days after treatment with cisplatin or vehicle control. Red blood cells were lysed and peripheral blood cells were stained for **(D)** VEGFR-1+CD45+ hematopoietic cells and **(E)** VEGFR-1+CD45+CXCR4+ hemangiocytes.

Supplemental Figure 5. Chemotherapy effects are specifically blocked by VEGFR-1 antibodies and these effects are independent of VEGFR-1 kinase signaling.

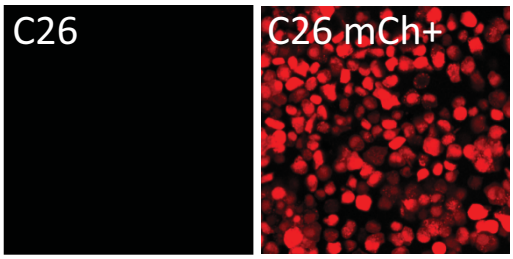
Mice were pretreated with cisplatin or vehicle control at day -4. At day -1, 800 μg DC101 or vehicle control was administered per mouse and at day 0, C26 cells were administered iv to BALB/c mice **(A)**, or B16F10 cells were administered to C57Bl/6 mice **(B)**. At day 13, lung colonies were analyzed by counting surface metastases. **(C)** C26 cells were plated and DC101 was added in a concentration of 50 μg/ml. MTT assays were performed on 3 following days to determine the proliferation rate compared to vehicle control.

Supplemental Figure 1.



Supplemental Figure 2.

A.



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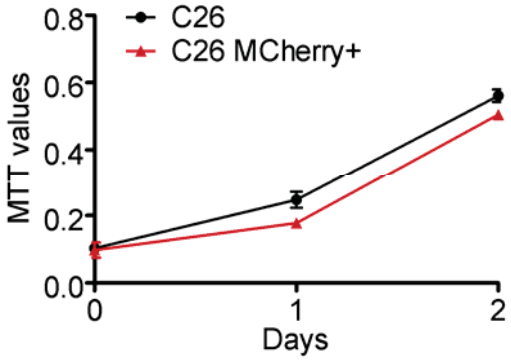

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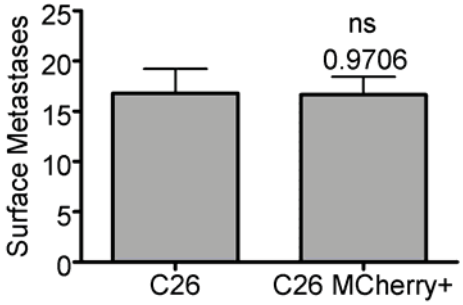

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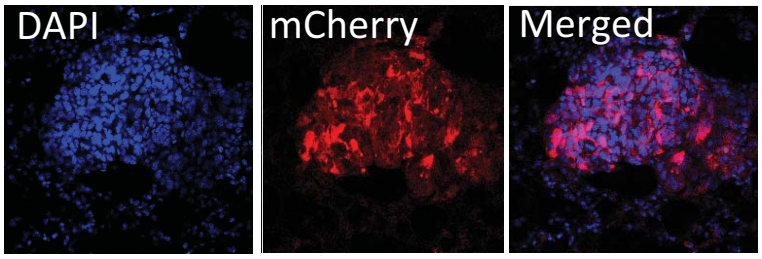
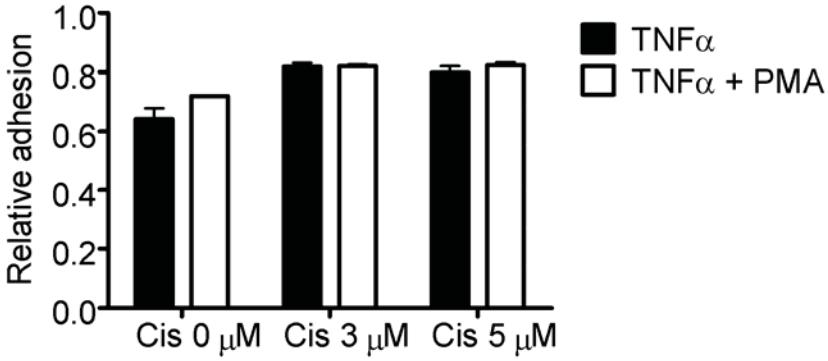


Supplemental Figure 3.

A.



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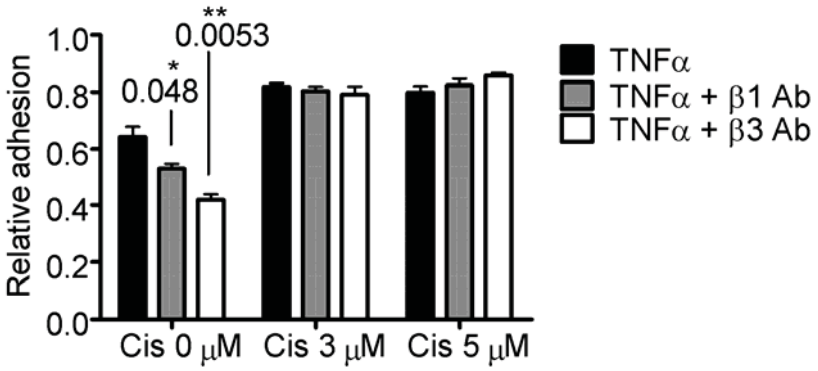

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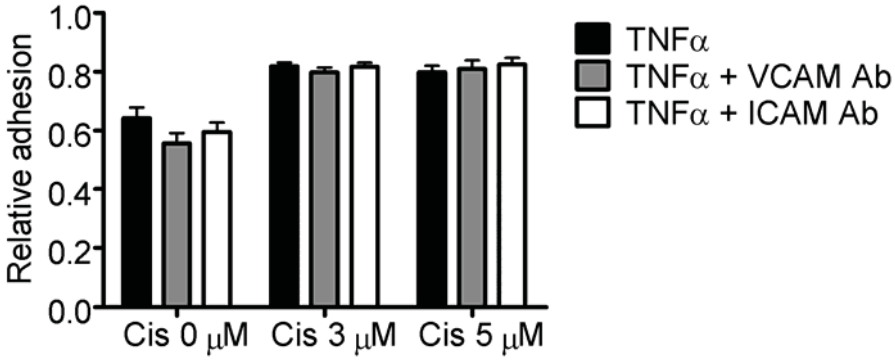
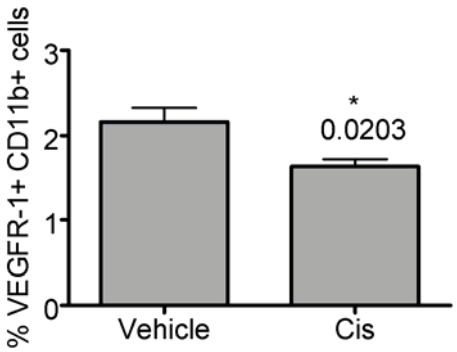


Supplemental Figure 4.

A.



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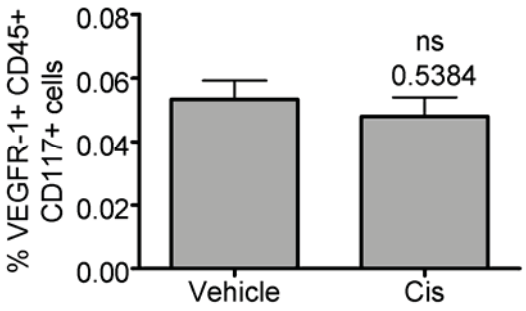

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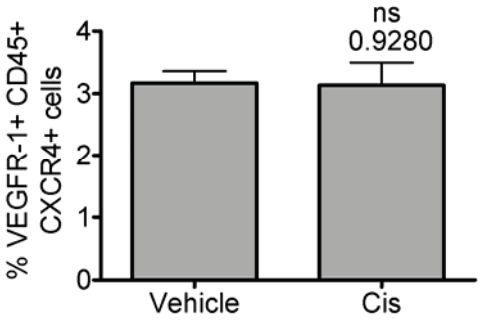

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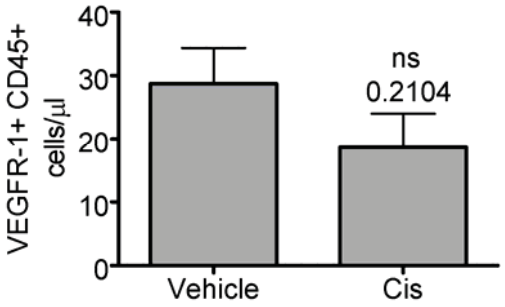

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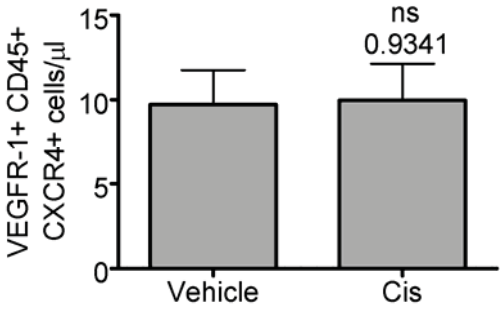

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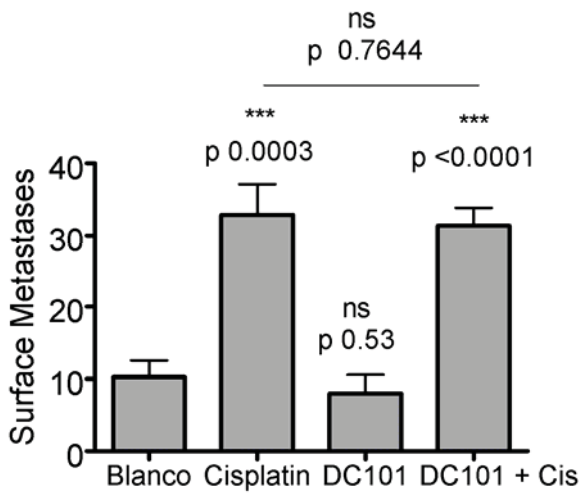
A.



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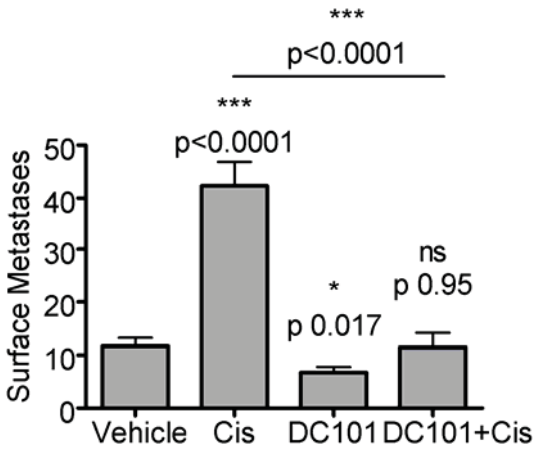

C.