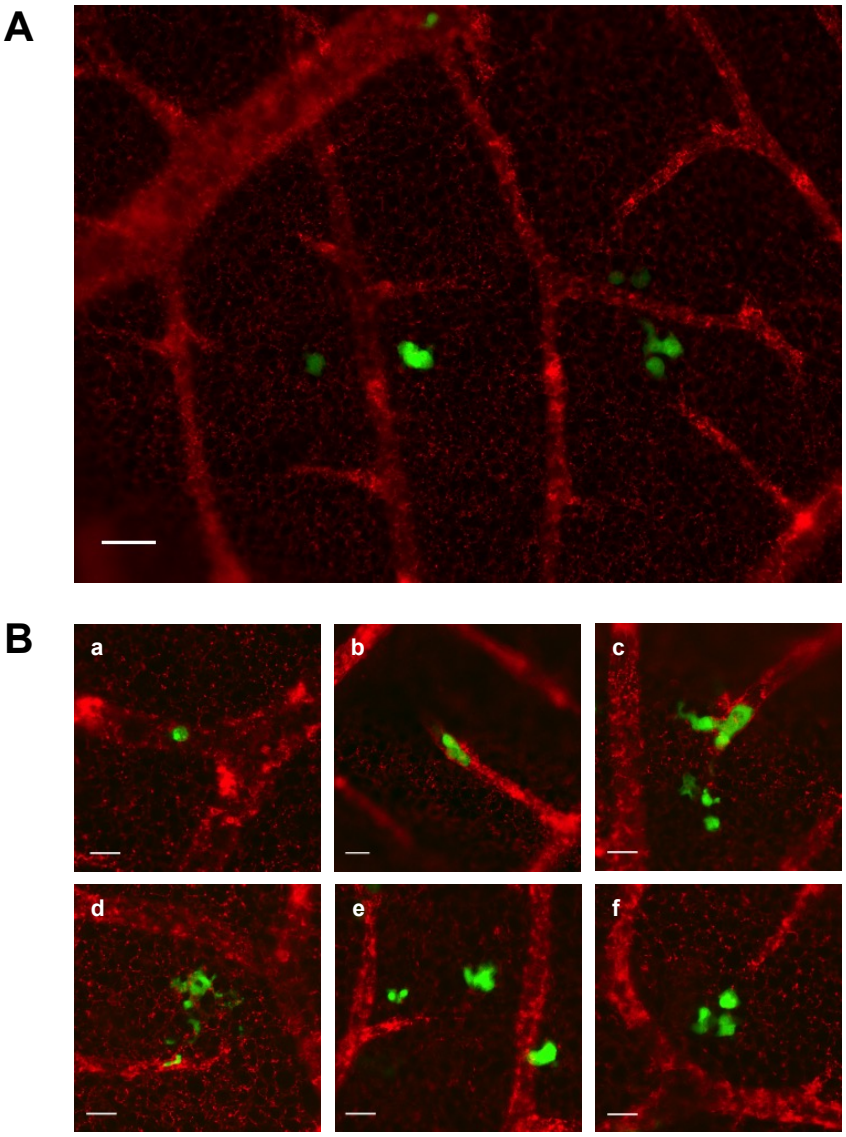

Figure S1. Immunofluorescent analysis of tumor cells following their intravasation at the primary tumor site in a spontaneous metastasis model.

(***A***) GFP-labeled HT-hi/diss cells were grafted on the CAM of 10 day-old chick embryos developing *in ovo* ($5x10^5$ cells per embryo). On day 5, the embryos were injected i.v. with Rhodamine-conjugated LCA to highlight the CAM vasculature (red). Primary tumors were excised and portions of the distal CAM were visualized in epifluorescent microscope. The areas in the distal CAM with occasional GFP-tagged cells were imaged at original magnification of 200X as 1-µm-thick optical z-stacks. These GFP-labeled cells represent intravasated tumor cells, which had disseminated *via* angiogenic blood vessels developed within the primary tumor, and then were rapidly arrested in the narrow capillary networks of the CAM. Scale bar, 25 µm.

(***B***) HT-hi/diss cells, which have entered the circulation at the primary tumor site and then were trapped in the CAM capillary system, could be seen as intact and viable single cells at different stages of active progression towards extravasation from the ectoderm capillary plexus of the CAM into the CAM mesoderm: (***a***) tumor cell still in the circulation; (***b***) a tumor cell has reached the tip of a terminal arteriole (that is branching into tiny capillaries of the ectoderm plexus); (***c***) tumor cell appears to be pushed out from the terminal arteriole; (***d***) tumor cell that just entered the ectoderm plexus, thereby filling the donut-shaped capillaries; (***e***) three tumor cells apparently extravasating from the ectoderm plexus into CAM mesenchyme; (***f***) two tumor cells in the CAM mesenchyme; one of the cells appears as undergoing cell division. Scale bars, 25 µm.

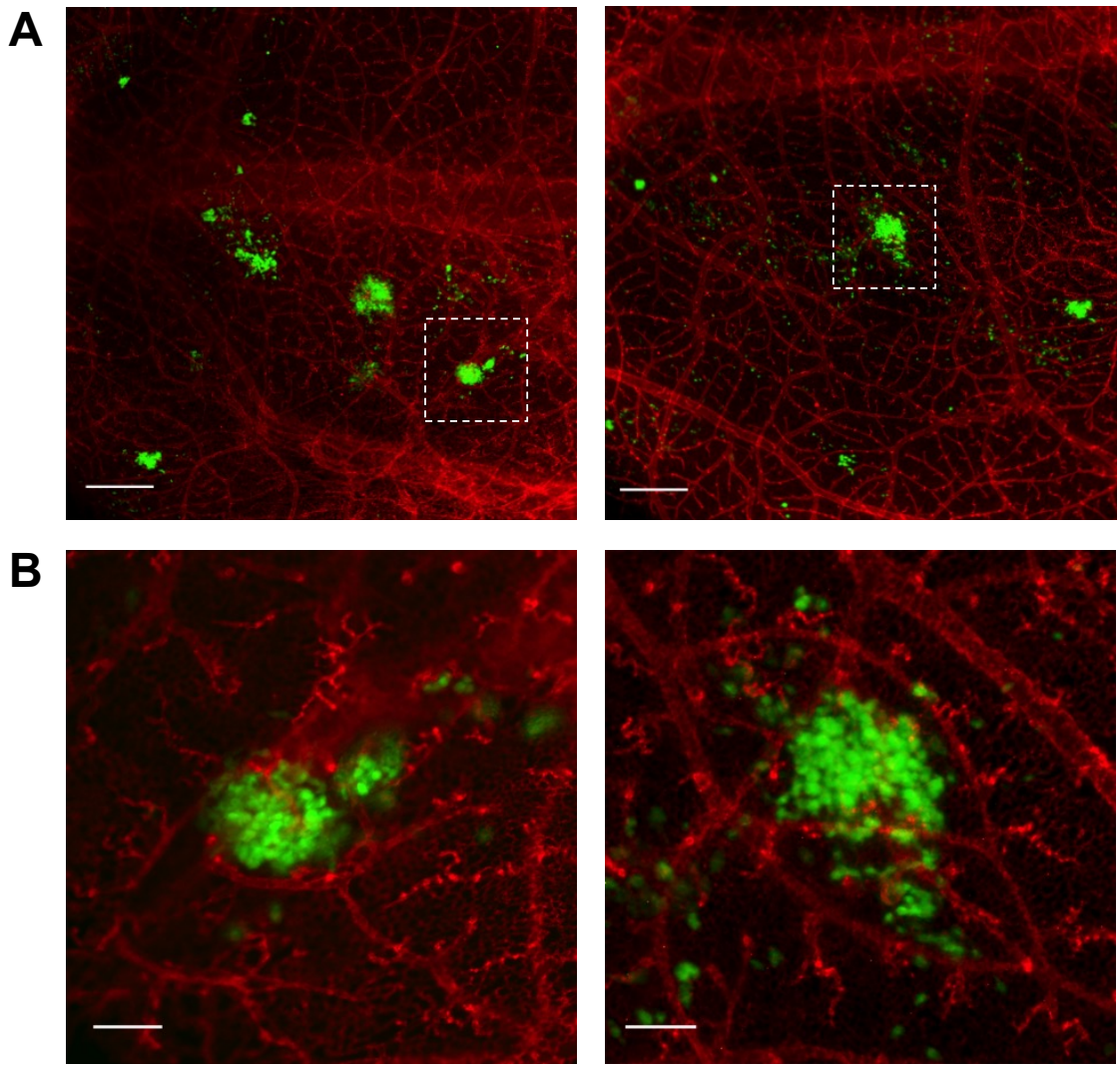

Figure S2. Immunofluorescent analysis of CAM tissue colonization in an experimental metastasis model.

GFP-tagged HT-hi/diss cells were inoculated intravenously into day 12-old chick embryos. Because of the particularities of the blood circulation in the avian embryo, intravenously inoculated cells are arrested in the CAM vasculature and therefore, GFP-tagged tumor cells could be visualized by epifluorescent microscopy against LCA-contrasted CAM tissue. Portions of the CAM were visualized in epifluorescent microscope 5 days after cell inoculations. Intravenously injected tumor cells formed multi-cellular colonies within the CAM stroma. This robust colony formation, characteristic of metastatic outgrowths, is in clear contrast to the single cell status of spontaneously intravasated cells arrested in the CAM vasculature (shown in Supplemental Figure S1).

(***A***) Original magnification, 40X. Scale bars, 500 μm.

(***B***) Individual colonies presented in (A) were additionally imaged at a higher magnification of 200X. Scale bars, 100 μm.

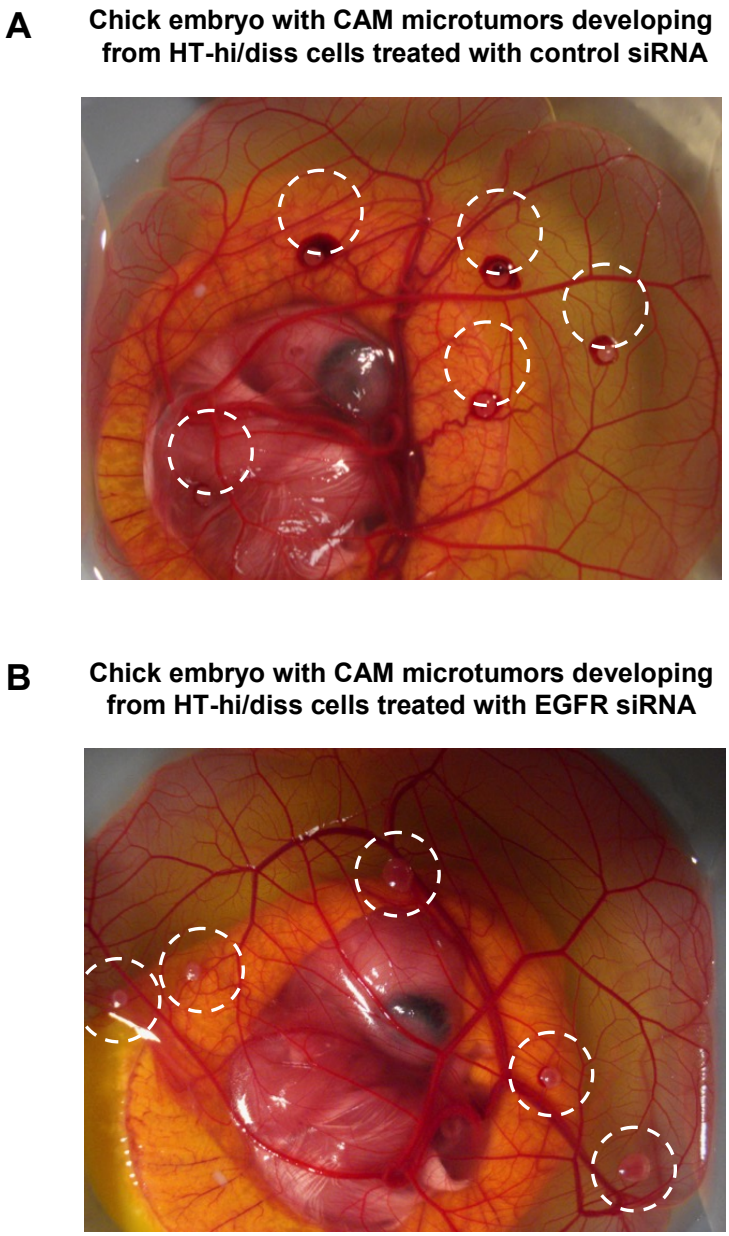

Figure S3. CAM microtumor model.

HT-hi/diss cells treated with control siRNA (***A***) or EGFR siRNA (***B***) were grafted as 10 µl-aliquots of collagen-containing cell mixtures on the CAM of 10 day-old chick embryos developing *ex ovo*. Within 5 days, both cell types gave rise to microtumors (outlined by white circles) of comparable size, allowing for quantification of intravasation rates by *Alu*-qPCR and quantitative analyses of microarchitecture and permeability of intratumoral vasculature in individual microtumors by epifluorescent microscopy. Note that control microtumors appear more hemorrhagic than tumors originating from EGFR-silenced cells, suggesting that EGFR regulates the levels of tumor angiogenesis and/or blood vessel permeability.

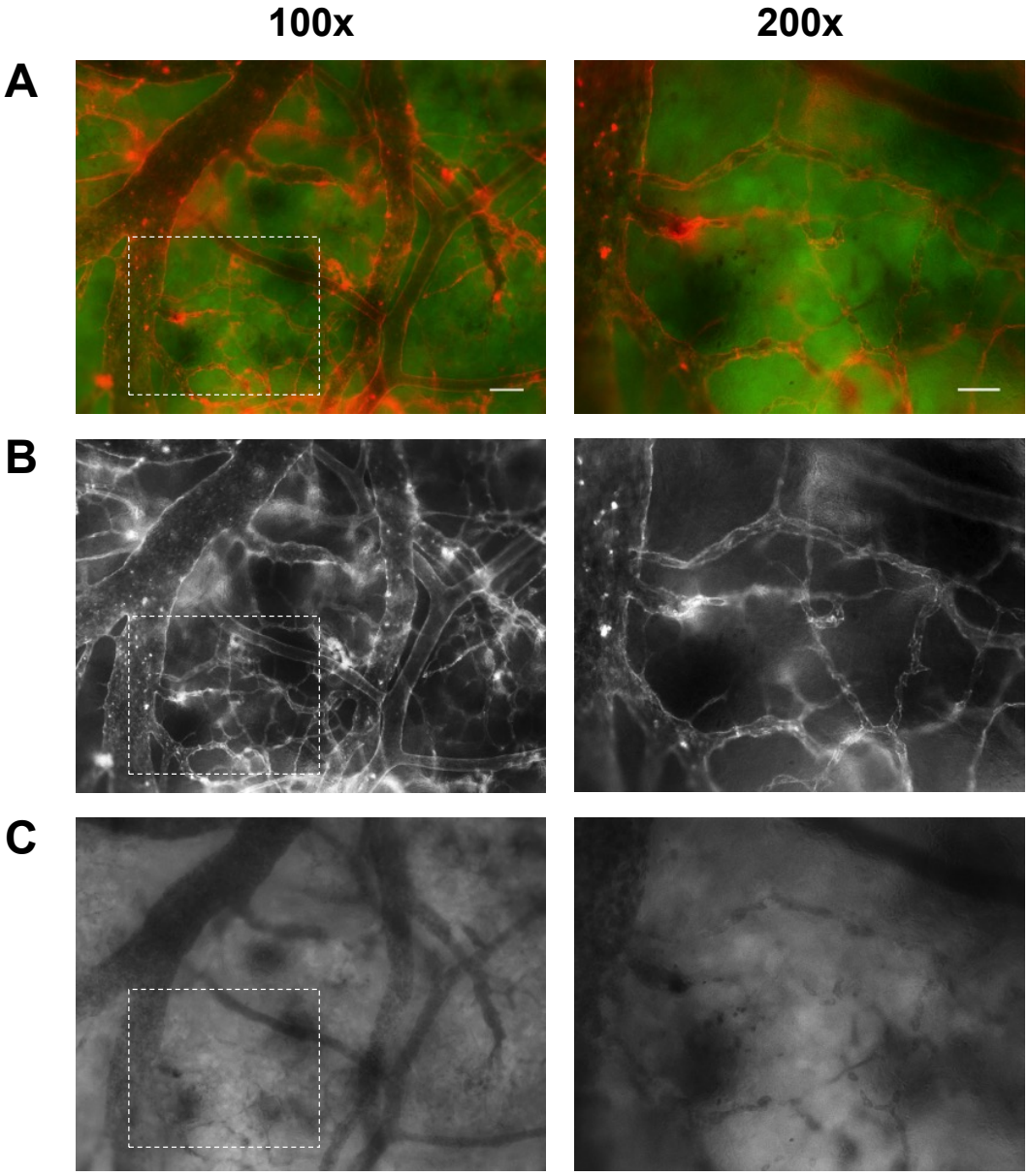

Figure S4. Immunofluorescent analysis of intratumoral vasculature in the CAM microtumor model.

Microtumors were initiated by grafting GFP-tagged HT-hi/diss cells on the CAM of 10 day-old embryos incubated *ex ovo* (shown in Supplementary Figure S3). Five days later, the CAM vasculature was highlighted with intravenously inoculated Rhodamine-conjugated LCA. Portions of the CAM with individual microtumors were excised and visualized without any fixation in epifluorescent microscope. Images of fluorescently green intratumoral areas of individual microtumors were acquired as 5 µm-thick z-stacks. Acquisition was performed independently for the red and green fluorescent signals.

Left, Presented are single optical z-planes of the inner portion of a microtumor acquired at original magnification of 100X. Scale bar, 50 µm. ***Right***, Intratumoral areas, indicated by dotted rectangles in the images on the left, were imaged at a higher magnification of 200X. Scale bar, 25 µm. ***A***, In the images on the top, green and red fluorescent signals, which originally were acquired independently in a black-and-white mode, were merged, confirming that presented red-fluorescent vasculature is indeed located intratumorally since it is surrounded by GFP-tagged tumor cells. ***B***, Images in the middle present red fluorescent signal only, allowing for a clear appreciation of the intratumoral vasculature microarchitecture and also allowing for quantitative analyses of blood vessel size. ***C***, Images at the bottom present the green fluorescent signal emitted by GFP-tagged tumor cells.