Supplemental Figure 1. Correlation of lymphatic markers from human metastatic melanoma samples. A. Immunofluorescence detection of PROX1^+Melan-A^− cells in primary human cutaneous melanoma. Nuclear PROX-1 staining consistent with lymphatic vessel staining (Melan-A^−, arrow) and cytoplasmic staining in Melan-A^+ cells (*). PROX1, red; Melan-A, green; DAPI, blue. Scale bar = 50µm. B. Correlation of lymphatic markers (LYVE1, PDPN, PROX1) with each other and with vascular endothelial growth factor genes (VEGFA, VEGFB, VEGFC, VEGFD). 266 metastatic cutaneous melanoma samples from the Broad Institute’s TCGA database. Pearsons correlation coefficient (r).
Supplemental Figure 2. The lymphatic score correlates with the lymphangiogenic growth factor VEGFC and not VEGF A,B or D. A. Distribution of lymphatic scores. B. Correlation of lymphatic score (LS) with VEGFC, PDPN, LYVE1, VEGFA, VEGFB, and VEGFD expression in 266 metastatic cutaneous melanoma samples. Pearson's correlation coefficient (r).
Supplemental Figure 3. Quantification of percent area of CD31 staining. A. Quantification of percent area of CD31⁺ pixels quantified over total image area from immunohistochemical staining in paraffin sections. Data represented as mean ± SEM, n=3, **p<0.05.
Supplemental Figure 4. K14-VEGFR3-Ig mice do not exhibit enhanced cutaneous fibrosis as compared to wildtype littermates. A. Picosirius Red staining was performed on skin taken from naive wildtype (WT) and K14-VEGFR3-Ig (TG) mice. Collagen fibers are stained red; scale bar = 100µm. B. Immunohistochemistry for α-SMA in naïve skin from WT and TG mice (scale bar = 50µm) and C. quantification of number of α-SMA+ cells per 0.04mm²; n=3. Data represented as mean ± SEM, n=3. D. Picosirius Red staining in B16F10 tumor implanted intradermal in WT and TG mice. Scale bar = 100µm; “T” marks tumor.
Supplemental Figure 5. Lymph node cellularity in wildtype and K14-VEGFR3-Ig mice. Brachial lymph node cellularity (naïve) in WT and TG mice, n=4. P-values were obtained with Student’s unpaired t test.
Supplemental Figure 6. Gating scheme for analysis of infiltrating immune populations by flow cytometry. Multi-color flow cytometry gating scheme to quantify immune populations in tumor, spleen and draining lymph node. Representative plots from the tumor of wildtype mouse.
Supplemental Figure 7. Homeostatic cutaneous immune populations in wildtype and K14-VEGF3-Ig mice. Naïve skin and spleens were digested and analyzed by flow cytometry for immune populations from wildtype (WT) and K14-VEGFR3-Ig (TG) mice. A. Total CD45<sup>+</sup> cells as a percent of live cells and B. CD3ε, CD11c and CD11b cells as a percent of CD45<sup>+</sup> cells are quantified from the skin and C and D. spleens of mice. E. Representative plots of Ly6c<sup>+</sup> and Ly6g<sup>+</sup> populations in spleens of naïve and tumor-bearing mice and F. quantification of % Ly6c<sup>+</sup>Ly6g<sup>-</sup> inflammatory monocytes in naïve spleens, n≥4. Quantification of tumor-bearing mice in Figure 3. Gated on CD45<sup>+</sup>CD11c<sup>-</sup>CD11b<sup>-</sup>F4/80<sup>-</sup> inflammatory monocytes.
Supplemental Figure 8

Supplemental Figure 8. Correlation between lymphatic involvement and intratumoral cytokine expression. A. Representative blot (above) and key (below) from cytokine array, n=7. Black squares indicate control IgG spots used for relative intensity calculations. Quantification presented in Figure 3. B. Representative western blots on tumor lysate and C. quantification of optical density normalized to WT (n=3); data are represented as mean ± SEM. P-values were obtained with Student’s unpaired t tests of values. *p<0.05, **p<0.01. D. Representative plots correlate expression of key cytokines (CCL2, TGFβ, MCSF, IL1B) with lymphatic score in 266 cutaneous metastatic melanoma samples from the Broad Institute TCGA database. Pearsons correlation coefficient (r).
Supplemental Figure 9. Chy mice demonstrate decreased dermal lymphatic vessel density and leukocytic infiltrate. C3BHA syngeneic breast carcinoma cells were injected intradermal (n=5) into ears of Chy mice harboring an inactivating mutation in the tyrosine kinase domain of VEGFR3. Lymphatic vessel density (LYVE1; 20x scale bar=100µm) and leukocytic infiltrate (CD45 and CD3ε; 40x scale bar=50µm) were evaluated by immunohistochemistry (left) and quantified as number per area (right). P-values were obtained with Student’s unpaired t tests of values. **p≤0.01 ***p<0.001.
Supplemental Figure 10. Inhibition of VEGFR3 signaling during tumor progression is insufficient to suppress local inflammation in wildtype mice. A. B16F10 tumors were implanted into wildtype mice and treated with either VEGFR3 neutralizing antibody (mF4-31C1, Eli Lilly and Company, 500 µg) or rat IgG isotype control on day 0, 3 and 6 to inhibit active VEGF-C signaling during tumor development. B. Fluid drainage was assessed by intratumoral injection of 70kDa FITC dextran and analysis of tumor draining lymph node 30 min later. (n=5) Relative numbers of tumor-associated C. CD45+ cell populations and D. inflammatory monocytes (CD11c-CD11b+Ly6c-Ly6g), quantified as a percent of live cells in the tumor. Relative numbers of splenic (E) CD45+ cell populations and F. inflammatory monocytes (CD11c-CD11b+Ly6c-Ly6g) (n=5). Data represented as mean ± SEM.