Figure S1. Characterization of the MT/ret mouse model. (A) Multiple malignant melanoma in the skin of MT/ret transgenic mice showed pronounced supply by blood vessels. Tumor cells of these mice metastasize to lymph nodes (100%) and spleen (80%; n = 74 mice). (B) MT/ret melanomas (n = 5 tumors of three mice) express the melanocyte-specific enzymes tyrosinase, TRP-1, and gp-100 in accordance with human melanoma. Primary antibodies are presented in green, and nuclei were counterstained by propidium iodide (red). (C) Immunofluorescence labeling of Lyve-1–expressing lymphatic vessels (green) localized in the connective tissue of tumor septs of MT/ret melanoma. Nuclei were stained in red. Colocalization of Lyve-1 and the endothelial marker CD31 (red) showed peritumoral localization of lymphatic vessels (n = 20 tumors of five mice). Representative pictures of more than five independently performed experiments are shown. Bars, 50 µm.
Figure S2. Effects of PTK/ZK on vessel formation in MT/ret melanoma. (A and C) Distribution analysis [in percentage] for the vascular network of MT/ret melanoma per mouse at the end of the prevention therapy (A; starting tumor-free, n = 10 and 9 vehicle- and PTK/ZK-treated mice) or at the end of the therapeutic intervention setting (C; starting tumor bearing) in vehicle- or PTK/ZK-treated mice (n = 10 mice per group of two independent experiments; ***, P ≤ 0.001). Error bars, mean ± SD. (B) Immunohistochemical assessment of the vascular bed of tumors, isolated after prevention therapy of vehicle- (top) or PTK/ZK- (bottom) treated mice using the endothelial cell marker CD31 (brown; n = 10 tumors/experimental group). (D) Vessel regression, detected by empty vessel sleeves, in preexistent tumors of high angiogenic potential after treatment of PTK/ZK, marked by arrowheads. Blood vessels were detected by CD31 (brown) and nuclei counterstained using Hematoxylin (n = 10 tumors of three mice). Representative images of more than five independent experiments are presented (B and D). Bars, 50 µm.
Figure S3. Effects of PTK/ZK on macrophage recruitment in MT/ret melanoma. (A) Immunofluorescence labeling of F4/80-expressing macrophages (green) recruited into high and low vascularized tumors marked by arrowheads. Blood vessels are indicated by dotted lines. Representative images of three independently performed experiments are presented (n = 10 high and 10 low angiogenic tumors of three mice). Bar, 50 µm. (B) Quantification for the number of F4/80-expressing macrophages per tumor area (in millimeters squared) in high and low angiogenic tumors of MT/ret transgenic mice treated with vehicle or PTK/ZK for therapeutic intervention. Data are representative of 10 tumors of three mice per experimental group analyzed in two separate experiments. Error bars, mean ± SD.