SUPPLEMENTARY INFORMATION

Supplementary Figures

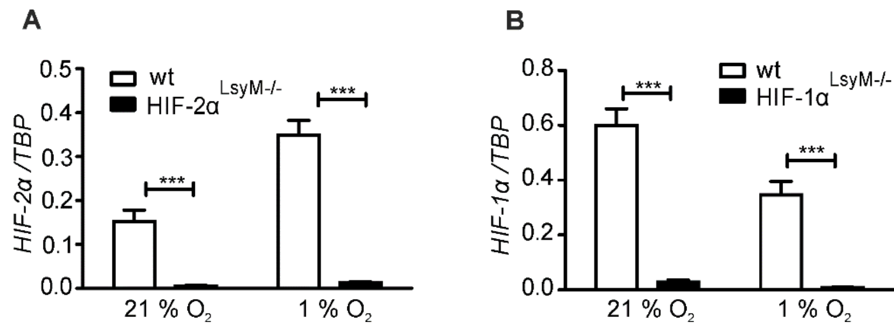

Figure S1 Validation of mice with a macrophage-specific HIF-1α or HIF-2α knockout. (A) *HIF-2α* and (B) *HIF-1α* mRNA expression in wt, HIF-2α-, or HIF-1α-depleted BMDMs was measured by qPCR and is given relative to *TBP*. Data were analyzed using two-way ANOVA with Bonferroni's correction and represent means ± SEM ($n = 5$; ***$p < 0.001$).

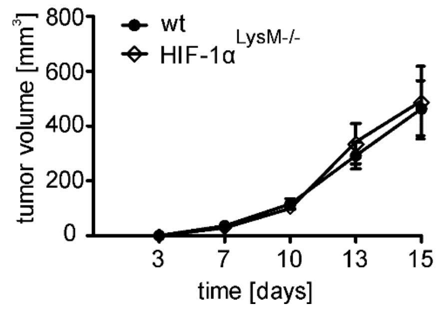

Figure S2 Effect of myeloid HIF-1α expression on breast tumor growth. 50,000 murine E0771 breast adenocarcinoma cells were injected subcutaneously into breast glands 3 and 8 of 10-12 weeks old female wt or HIF-1α$^{LysM-/-}$ mice. Tumor size was determined every second day (n = 7). Data were analyzed using a two-way ANOVA with Bonferroni's correction and represent means ± SEM.

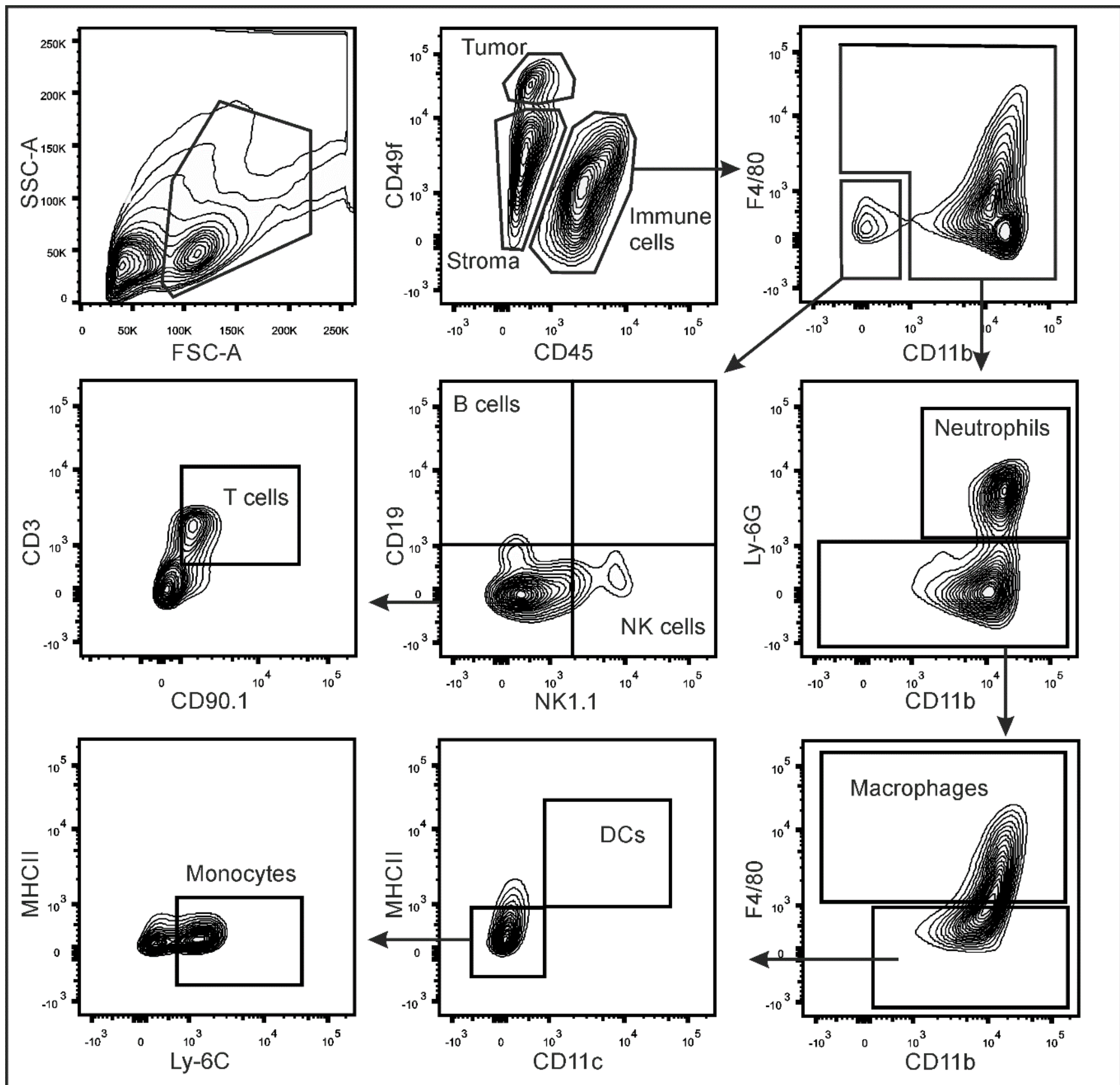

Figure S3 Gating strategy to identify different immune cell populations. Single cell suspensions of dissociated tumors were discriminated by gating SSC-H and FSC-A. Immune cells were distinguished from tumor and stroma cells by positive staining for CD45 and negative to low positive staining for CD49f. Lymphocytes were defined as both F4/80 and CD11b negative cells and further characterized as natural killer (NK) cells (NK1.1^{+}, CD19^{-}), B cells (NK1.1^{-}, CD19^{+}), and T cells (NK1.1^{-}, CD19^{-}, CD3^{+}, CD90.1^{+}). Myeloid cells were additionally defined as neutrophils (Ly-6G^{+}, CD11b^{+}), macrophages (F4/80^{+}, CD11b$^{-/+}$), DCs (CD11c^{+}, MHCII^{+}) and monocytes (Ly-6C^{+}, MHCII^{-}). The contour blots show the gating from a representative wt tumor.

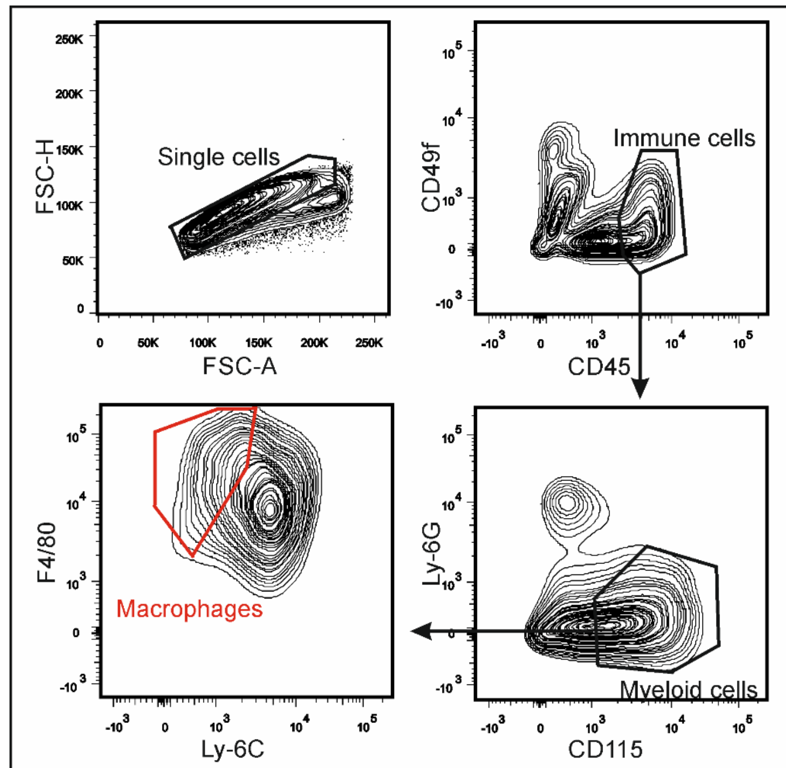

Figure S4 Sorting strategy to isolate TAMs for RNA-seq. Single cells from dissociated tumors were defined by discriminating cell doublets gating FSC-H and FSC-A. Immune cells were distinguished from tumor and stroma cells by a positive staining for CD45 and a low positive to negative staining for CD49f. To obtain myeloid cells, $CD115^{+}$ cells were gated negative for Ly6G to exclude neutrophils. Within the myeloid cell population, monocytes, characterized by $Ly6C^{+}$ and $F4/80^{-}$, were excluded and TAMs ($F4/80^{+}$ and $Ly6C^{low/-}$) were sorted. The contour blots show the gating from a representative wt tumor.

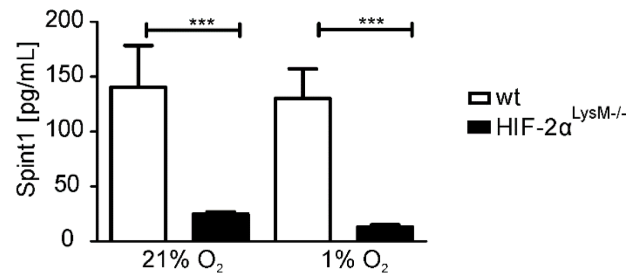

Figure S5 Induction of Spint1 in wt vs. HIF-2α knockout BMDMs. wt and HIF-2α$^{LysM-/-}$ BMDMs were incubated for 7 days under 21% or 1% O_2, before Spint1 protein amounts were assessed in the cell lysates by ELISA. Data were analyzed using two-way ANOVA with Bonferroni's correction and represent means ± SEM (n = 4; ***$p < 0.01$).

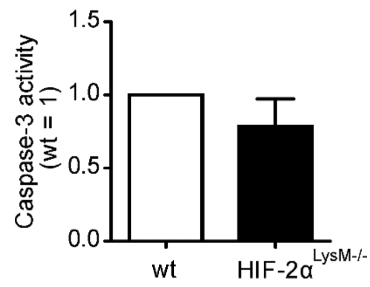

Figure S6 Cell death of HIF-2α$^{LysM-/-}$ BMDMs under hypoxia. Caspase-3 activity of HIF-2α$^{LysM-/-}$ BMDMs normalized to wt BMDMs. Data were analyzed using two-way ANOVA with Bonferroni's correction and represent means ± SEM (n = 5).

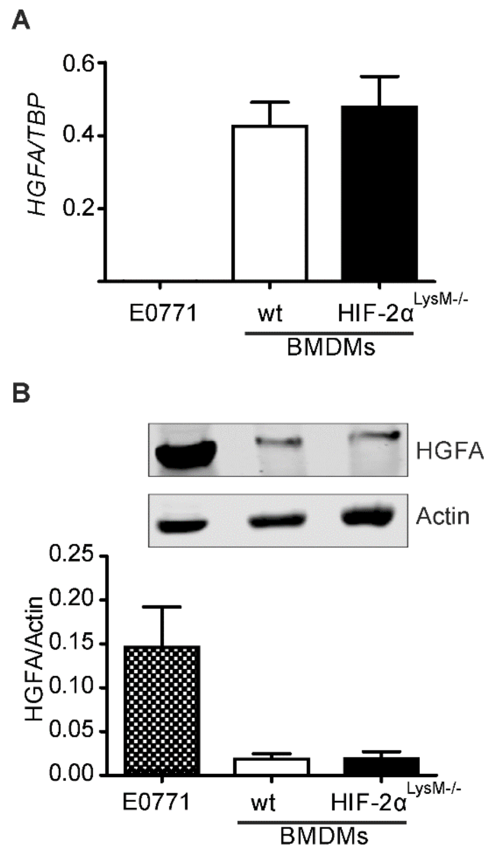

Figure S7 HGFA expression in tumor cells, BMDMs and TAMs. (A) *HGFA* mRNA expression was analyzed in E0771 tumor cells and hypoxic BMDMs isolated from wt and HIF-2α$^{LysM-/-}$ mice by qPCR and is given relative to *TBP*. (B) HGFA protein was measured in hypoxic BMDMs isolated from wt and HIF-2α$^{LysM-/-}$ mice by Western blot and is given relative to Actin. Data represent means ± SEM (n = 3).

Supplementary Methods

Viability assay

BMDMs were isolated and differentiated as described above. After 7 days incubation at 1% O_2 caspase-3 activity was measured, according to the manufacturer's instruction using the Ac-DEVD-AMC Caspase-3 Fluorogenic Substrate (BD Pharmingen, Heidelberg, Germany).

Western blot analysis

E0771 cells were harvested in 50 µl lysis-buffer (4% SDS, 150 mM NaCl and 100 mM Tris/HCl supplemented with 1x Protease Inhibitor Mix, 1 mM DTT and 1x PhosphoStop, pH 7.4). Protein content was determined by a Lowry assay (BioRad, Munich, Germany) and 80 µg protein was loaded on a 7.5% SDS gel. Proteins were transferred to a nitrocellulose membrane (Millipore, Darmstadt, Germany) using a Trans-Blot Turbo blotting system (BioRad). Membranes were blocked in 5% BSA in TBS-T (50 M Tris/HCl, 140 mM NaCl, 0.05 % Tween-20, pH 7.2) for 1 hour and afterwards incubated with primary antibodies against HGFA (1:1000, LSBio, WA, USA) and Actin (1:5000, Sigma-Aldrich) overnight at 4°C. Detection was performed using the appropriate secondary antibodies on an Odyssey infrared imaging system (LI-COR, Biosciences, Bad Homburg, Germany). Image Studio Digits 5.0 (LI-COR) software was used for quantification.