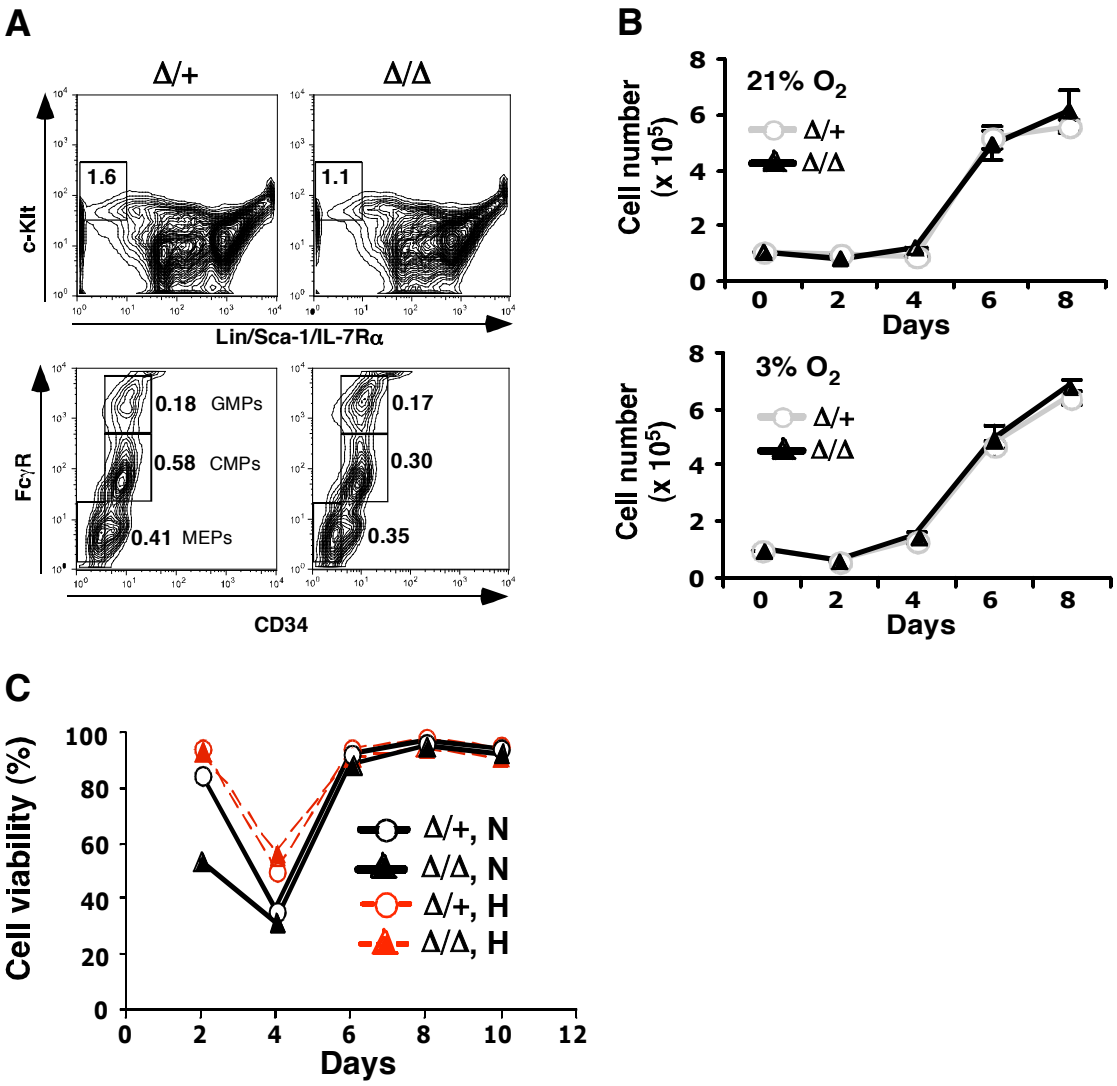
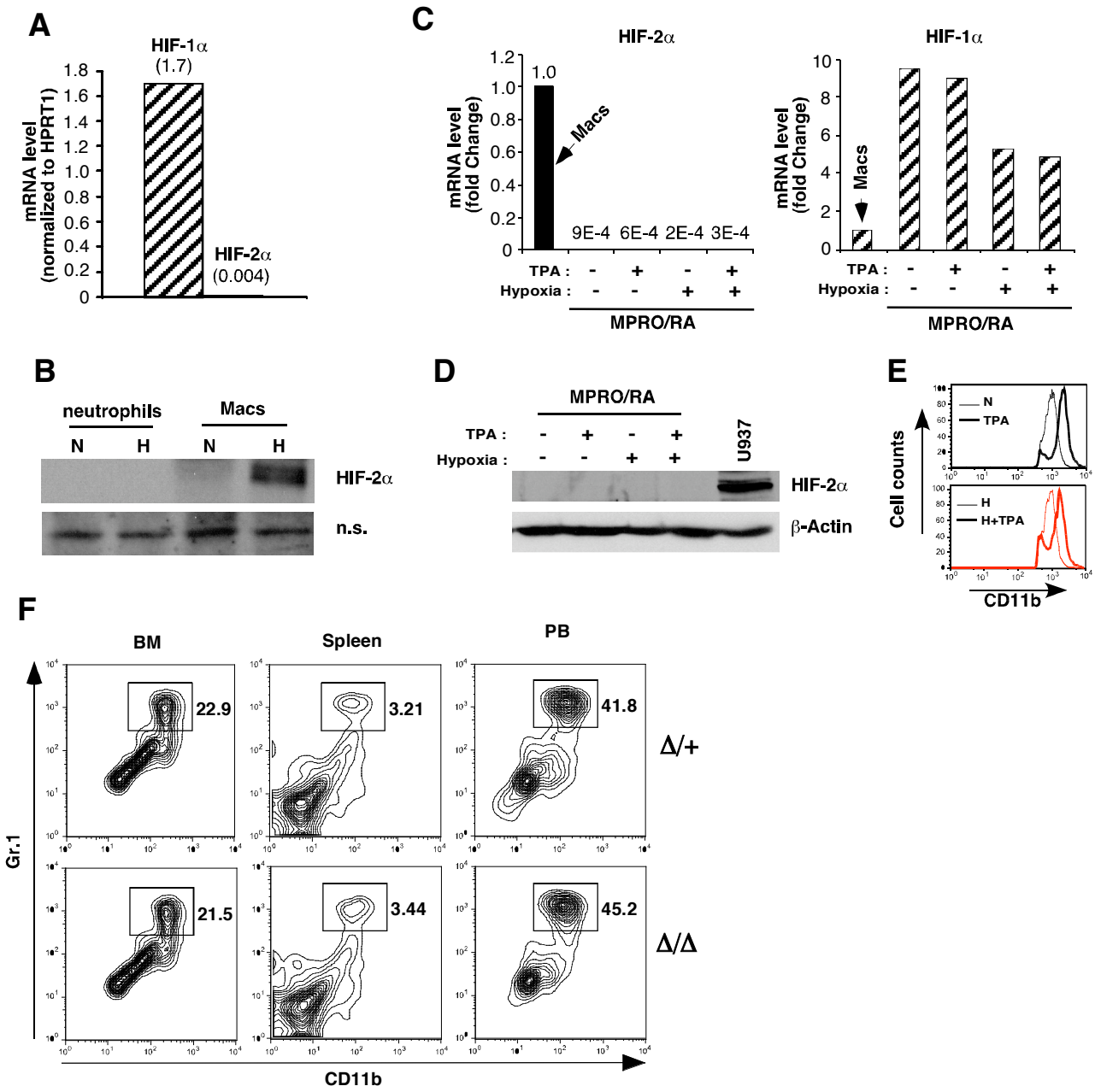
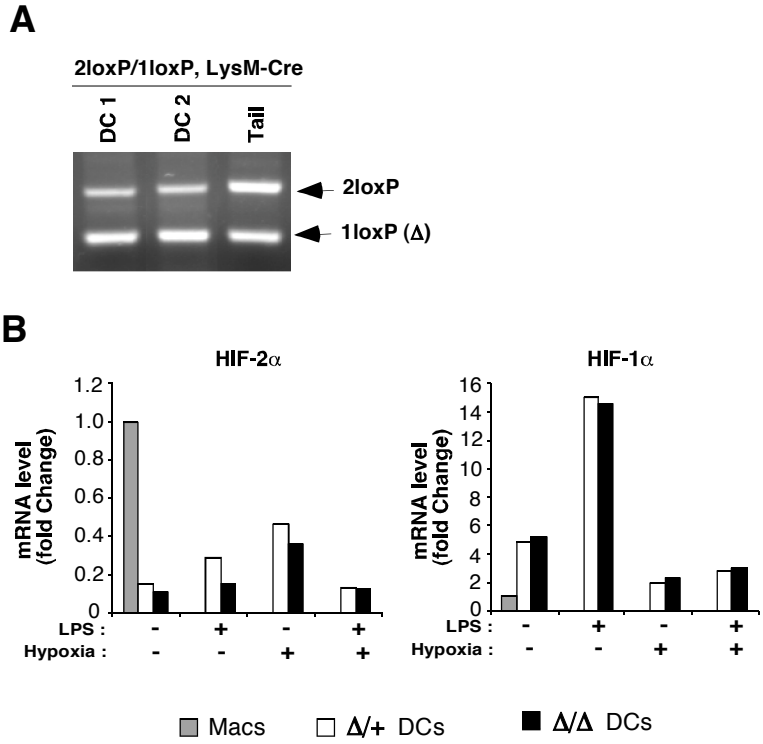


Supplemental Figure S1. Macrophage maturation, proliferation and survival are not dependent on HIF-2α. (A) Myeloid progenitors are not influenced by HIF-2α. The IL-7Rα$^-$Lin$^-$Sca-1$^-$c-Kit$^+$ fraction (gated, top panel) was subdivided into CMPs (FCγRII/III^{lo}CD34$^+$), GMPs (FCγRII/III^{hi}CD34$^+$), and MEPs (FCγRII/III$^{lo/-}$CD34$^-$) based on their CD34 and FCγRII/III expression. Representative panels of FACS analyses are shown. (B) Normal proliferation of *Hif-2α$^{Δ/Δ}$* BMDMs. BMDMs treated with normoxia (21% O_2) and hypoxia (3.0% O_2) and their proliferation was determined by counting cell numbers every two days for eight days. (C) Normal survival of *Hif-2α$^{Δ/Δ}$* BMDMs. BMDMs were placed under normoxia (21% O_2) and hypoxia (0.5% O_2) and their viability was analyzed by propidium iodide uptake assay.



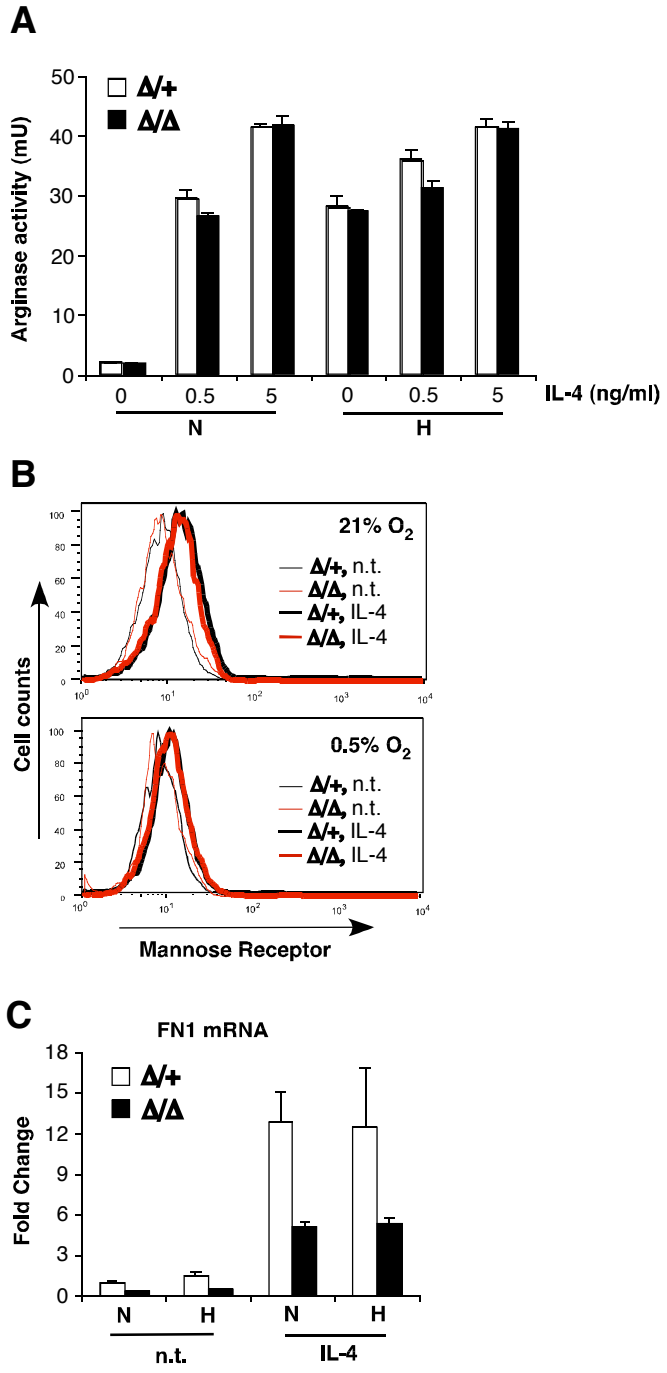
Supplemental Figure S2. Neuthophils do not express HIF-2α. (A) Expression of HIF-1α and HIF-2α in bone marrow-derived mouse neutrophils was evaluated at normoxia and relative mRNA levels were normalized to that of endogenous HPRT1. (B) HIF-2α protein stabilization in bone marrow-derived mouse neutrophils was compared to that in BMDMs exposed to hypoxia for 3hrs. n.s.: non-specific band as a loading control. (C) MPRO cells were differentiated for 3 days in the presence of RA (10 μM). HIF-2α and HIF-1α mRNA level was determined by QRT-PCR following the stimulation with TPA (100 ng/ml) for 15 min. (D) HIF-2α protein level in MPRO cells was evaluated by Western blotting following TPA treatment. (E) Surface expression of CD11b on MPRO cells was analyzed by flow cytometry following TPA treatment. (F) Normal development of neutrophils in *Hif2a*$^{\Delta/\Delta}$ mice. Neutrophils in the bone marrow, spleen and peripheral blood were quantified by immunostaining for CD11b in combination with Gr.1. N: normoxia (21% O_2); H: hypoxia (0.5% O_2); Macs: macrophages.

Imtiyaz et al., Fig. S3

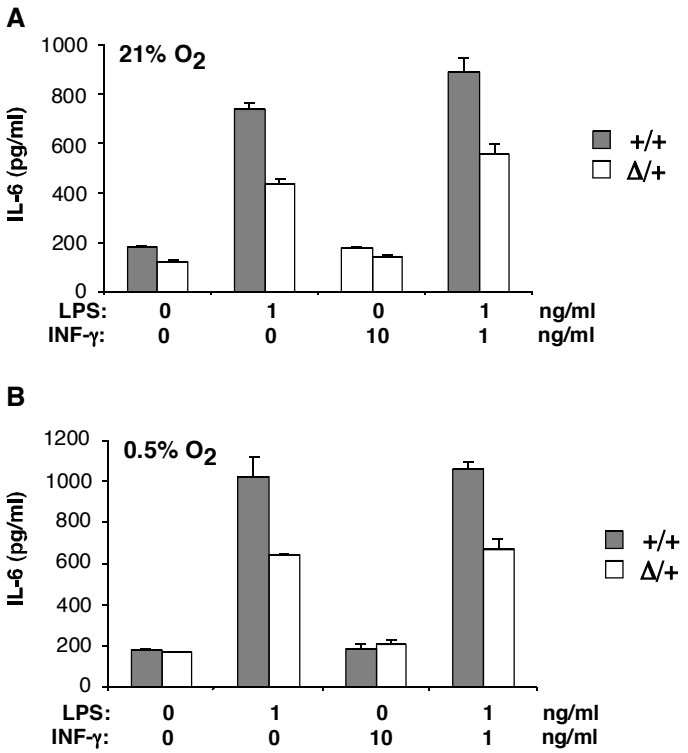


Supplemental Figure S3. HIF-2α is still expressed in *Hif2a*$^{\Delta/\Delta}$ dendritic cells. (A) Insufficient deletion of the *Hif-2α* floxed (i.e. 2loxP) allele. DNA was isolated from differentiated bone marrow-derived dendritic cells and subjected to PCR analysis. DC1 and DC2 indicate primary dendritic cells generated from two independent mice with *Hif2a*$^{\Delta/\Delta}$ genotype, Tail DNA obtained from *Hif2a*$^{\Delta/\Delta}$ mice was used as a control. (B) Dendritic cells were treated with LPS (10 ng/ml) for 24 hrs under normoxia and hypoxia, and mRNAs of HIF-2α and HIF-1α was quantified by QRT-PCR. (C) HIF-2α protein level in dendritic cells was evaluated by Western blotting following LPS treatment. Macs: macrophages; DCs: dendritic cells.

Imtiyaz et al., Fig. S4



Supplemental Figure S4. M2 responses of *Hif2a*$^{\Delta/\Delta}$ macrophages. (A) BMDMs were treated with recombinant IL-4 for 36 hrs a concentrations indicated. Arginase activity was measured using an isonitrosopropiophenone (ISPF)-based method. (B) Mannose receptor expression was determined by flow cytometry following 60 hrs of IL-4 stimulation. (C) FN1 expression was evaluated by QRT-PCR following 24 hrs of IL-4 stimulation. IL-4 concentration used for (B) and (C) is 5 ng/ml. N: normoxia; H: hypoxia.



Supplemental Figure S5. IL-6 production of M1-stimulated *Hif2a*$^{+/+}$ and *Hif2a*$^{\Delta/+}$ BMDMs under normoxia (A) and hypoxia (B) was determined by ELISA.

Supplemental Table S1: Characterization of HCC tumors in HIF-2α LysM-Cre mice

		Δ/+	Δ/Δ	n value (Δ/+, Δ/Δ)	p value (t-test)
Tumor number*		23.2 ± 2.67	21.1 ± 4.22	(27, 22)	0.666
Tumor area (% tumor/liver area)		21.6 ± 5.54	20.8 ± 5.34	(21, 21)	0.910
Blood vessel number**	**Luminized**	1.7E-05 ± 3.04E-06	1.9E-05 ± 3.01E-06	(15, 15)	0.509
	Non-luminized	4.9E-06 ± 7.81E-07	5.0E-06 ± 8.64E-07	(15, 15)	0.892

* Total tumor number per liver section

** Blood vessel number per unit tumor area