Supplementary Methods

Animals

HIF-1$\alpha^{fl/fl}$ LysM-Cre and control mice were crossed with mice expressing the polyoma virus middle T oncoprotein (PyMT) under the Mouse Mammary Tumor Virus (MMTV) promoter,[1] back-crossed into the C57BL/6 background for at least six generations. Breast tumors were extracted after 20 weeks.

Biotinylation of primary antibodies

For intracellular detection of transcription factors, monoclonal anti-mouse Id2 (B31-1, BD Biosciences) and monoclonal anti-human/mouse HIF-1α (MAB1935, R&D Systems, Wiesbaden, Germany) were biotinylated using LYNX rapid biotin (type 1) conjugation kits (AbD Serotec, Düsseldorf, Germany) following the manufacturer's instructions to obtain a final antibody concentration of 0.2 mg/ml. Successful biotinylation was validated upon incubation with streptavidin-PE-CF594 (BD Biosciences) by analyzing binding to antibody-capturing CompBeads (BD Biosciences) using flow cytometry.

Flow cytometry and FACS sorting

For fluorescence-activated cell sorting (FACS), single cell suspensions were generated from solid tissues by digestion with 3 mg/ml Collagenase IA (Sigma, Steinheim, Germany), 1 U/ml DNAse I (Promega, Mannheim Germany) in 50% DMEM for 30 min at 37°C, followed by processing with BD Biosciences' MediMachine, red blood cell (RBC) lysis and filtering through a 70 µm nylon mesh (BD Biosciences). Whole blood was subjected to RBC lysis only. Cells were transferred to FACS tubes, non-specific antibody binding to FC-γ receptors was

blocked with Mouse BD Fc Block (BD Biosciences) for 20 min on ice. This was followed by incubation with individual antibody cocktails for 30 min on ice. Directly before sample acquisition, Flow-Count Fluorospheres (Beckman-Coulter, Krefeld, Germany) were added to determine absolute cell counts. For intracellular detection of ID2 and HIF-1α expression, cells were harvested by centrifugation and fixed for 10 min on ice using Cytofix/Cytoperm buffer (BD Biosciences), followed by washing and permeabilization for at least 5 min using Perm/Wash buffer (BD Biosciences). Next, 20 ng/ml unlabelled streptavidin were added in Perm/Wash buffer for 30 min on ice followed by washing and addition of streptavin-PE-CF594 (BD Biosciences) and the biotinylated antibodies for at least 60 min on ice. Samples were acquired with a LSRII/Fortessa flow cytometer (BD Biosciences, Heidelberg, Germany) and analyzed using FlowJo software 7.6.1 (Treestar, Ashland, OR, USA). All antibodies and secondary reagents were titrated to determine optimal concentrations. Antibody-capturing CompBeads (BD Biosciences) were used for single-color compensation to create multi-color compensation matrices. For gating, fluorescence minus one (FMO) controls were used. The instrument calibration was controlled daily using Cytometer Setup and Tracking beads (BD Biosciences).

For FACS analysis of Flt3-L bone marrow (BM) cultures, cells were stained with the following antibodies: anti-CD11c-AlexaFluor700 (HL3, BD), anti-CD172a-FITC (P84, BD), anti-CD135-APC (A2F10.1, BD), anti-CD8-eFluor650 (53-6.7, eBioscience), anti-CD11b-eFluor605 (M1/70, eBioscience), anti-CD115-PE (AF598, eBioscience), anti-CD117-APC-eFluor780 (2B8, eBioscience) anti-B220-VioBlue (RA3-6B2, Miltenyi Biotec), anti-SiglecH-PerCP-Cy5.5 (551, BioLegend), anti-MHCII-PE-Cy7 (M5/114.15.2, BioLegend), anti-CD24-BrilliantViolet421 (M1/69, BioLegend) and a cocktail of FITC-labeled antibodies to stain mature lineage cells consisting of anti-

CD127 (A7R34, eBioscience), anti-NK1.1 (PK136, BioLegend), anti-Gr-1 (RB6-8C5, Immunotools), anti-CD3 (145-2C11, Immunotools), anti-CD4 (PJP6, Immunotools), anti-CD19 (PeCa1, Immunotools), anti-Ter119 (Ter119, Immunotools). Dead cells were excluded with 7-AAD. MDP, CDP, pre-cDC and pDC or cDC and macrophages were identified using separate antibody panels.

For FACS analysis of bone marrow single cell suspensions, cells were stained with the following antibodies: anti-CD11c-AlexaFluor700 (HL3, BD), anti-CD135-APC (A2F10.1, BD), anti-CD8-eFluor650 (53-6.7, eBioscience), anti-CD11b-eFluor605 (M1/70, eBioscience), anti-CD115-PE (AF598, eBioscience), anti-CD117-APC-eFluor780 (2B8, eBioscience) anti-B220-VioBlue (RA3-6B2, Miltenyi Biotec), anti-SiglecH-PerCP-Cy5.5 (551, BioLegend), anti-MHCII-PE-Cy7 (M5/114.15.2, BioLegend) and a cocktail of FITC-labeled antibodies to stain mature lineage cells consisting of anti-CD127 (A7R34, eBioscience), anti-NK1.1 (PK136, BioLegend), anti-Gr-1 (RB6-8C5, Immunotools), anti-CD3 (145-2C11, Immunotools), anti-CD4 (PJP6, Immunotools), anti-Ter119 (Ter119, Immunotools). Dead cells were excluded with 7-AAD.

For FACS analysis of spleen cell suspensions or RBC-depleted blood the following antibodies were used: anti-CD11c-AlexaFluor700 (HL3, BD), anti-CD172a-FITC (P84, BD), anti-CD135-APC (A2F10.1, BD), anti-CD3-PE-Cy5 (17A2, BD), anti-CD8-eFluor650 (53-6.7, eBioscience), anti-CD11b-eFluor605 (M1/70, eBioscience), anti-CD115-PE (AF598, eBioscience), anti-CD117-APC-eFluor780 (2B8, eBioscience) anti-B220-VioBlue (RA3-6B2, Miltenyi Biotec), anti-SiglecH-PerCP-Cy5.5 (551, BioLegend), anti-MHCII-PE-Cy7 (M5/114.15.2, BioLegend), anti-CD24-BrilliantViolet421 (M1/69, BioLegend).

For FACS analysis of PyMT tumor single cell lysates the following antibodies were used: anti-CD45-V450 (30-F11, BD), anti-CD11c-AlexaFluor700 (HL3, BD), anti-Ly6C-PerCP-Cy5.5 (AL-21, BD), anti-CD11b-eFluor605 (M1/70, eBioscience), anti-MHCII-APC (Miltenyi Biotec), anti-CD317/120G8-FITC (120G8, Dendritics).

For FACS sorting of MDP, CDP and CLP from lineage$^-$ cell-enriched BM the following antibodies were used: anti-CD11c-AlexaFluor700 (HL3, BD), anti-CD135-APC (A2F10.1, BD), anti-CD8-eFluor650 (53-6.7, eBioscience), anti-CD11b-eFluor605 (M1/70, eBioscience), anti-Sca-1-eFluor605 (D7, eBioscience), anti-CD115-PE (AF598, eBioscience), anti-CD117-APC-eFluor780 (ACK2, eBioscience) anti-B220-VioBlue (RA3-6B2, Miltenyi Biotec), anti-MHCII-PE-Cy7 (M5/114.15.2, BioLegend), anti-CX3CR1-PerCP (ACNT01, R&D Systems) and a cocktail of FITC-labeled antibodies to stain remaining mature cells consisting of anti-CD127 (A7R34, eBioscience), anti-NK1.1 (PK136, BioLegend), anti-Gr-1 (RB6-8C5, Immunotools), anti-CD3 (145-2C11, Immunotools), anti-CD4 (PJP6, Immunotools), anti-Ter119 (Ter119, Immunotools).

Supplementary tables

Table S1. Primers for quantitative real-time PCR

Primer	**Sequence, 5' → 3'**
Murine beta-actin forward	ccc tct gaa ccc taa ggc ca
Murine beta-actin reverse	ggg aca aca cag cct gga tg
Mouse HIF-1α-exon2 forward	agt cta gag atg cag caa gat ctc ggc
Mouse HIF-1α reverse	ctt cca cgt tgc tga ctt ga
Mouse PU.1 forward	ccc gga tgt gct tcc ctt at
Mouse PU.1 reverse	tcc aag cca tca gct tct cc
Mouse TBP forward	ctg acc act gca ccg ttg cca
Mouse TBP reverse	gac tgc agc aaa tcg ctt ggg a

Supplementary Figures and Figure Legends

Figure S1

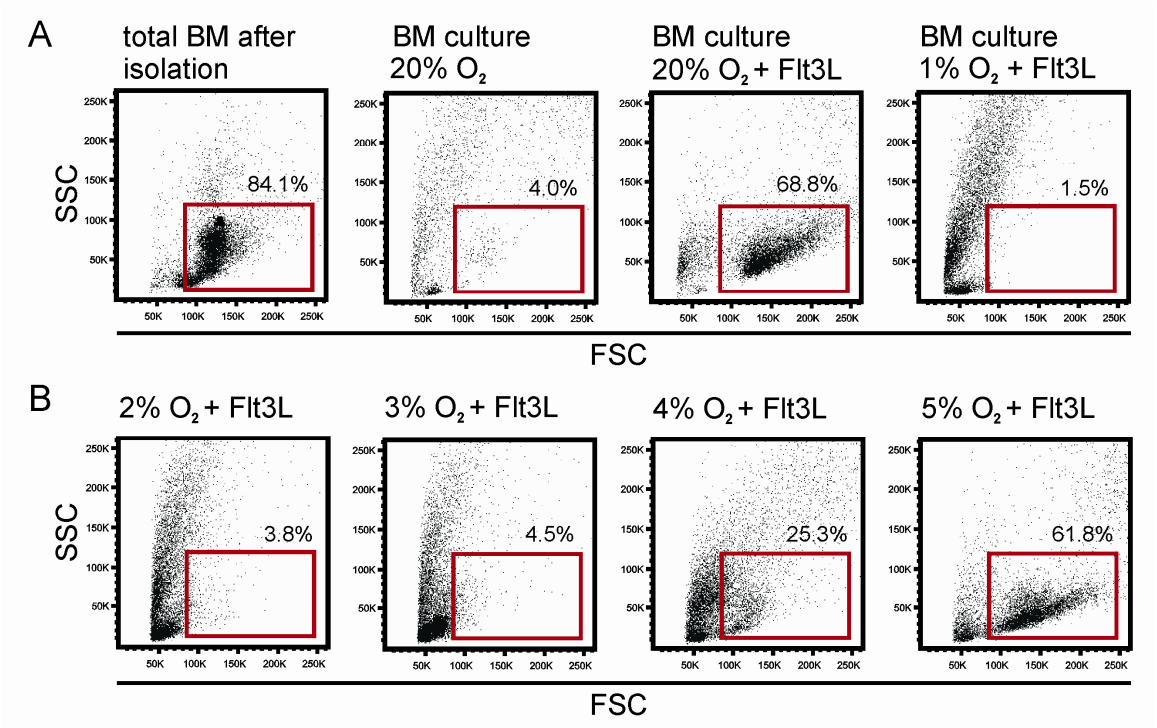

Figure S1. Impact of different oxygen levels on Flt3-L-induced DC generation from total bone marrow *in vitro*. (A,B) Bone marrow single cell suspensions of either wildtype mice were generated. 2 x 10^6 cells per ml were seeded in ultra-low attachment plates. (A) Cells were incubated with or without 200 ng/ml recombinant murine Flt3-L at 20% or 1% O_2 for 8 d. (B) Cells were cultured with 200 ng/ml Flt3-L at 2-5% O_2 for 6 d. Representative FACS traces show FSC *versus* SSC profile of expanded populations (the DC population expanded with Flt3-L under normoxia is indicated by the red square), representative of at least 3 independent experiments.

Figure S2

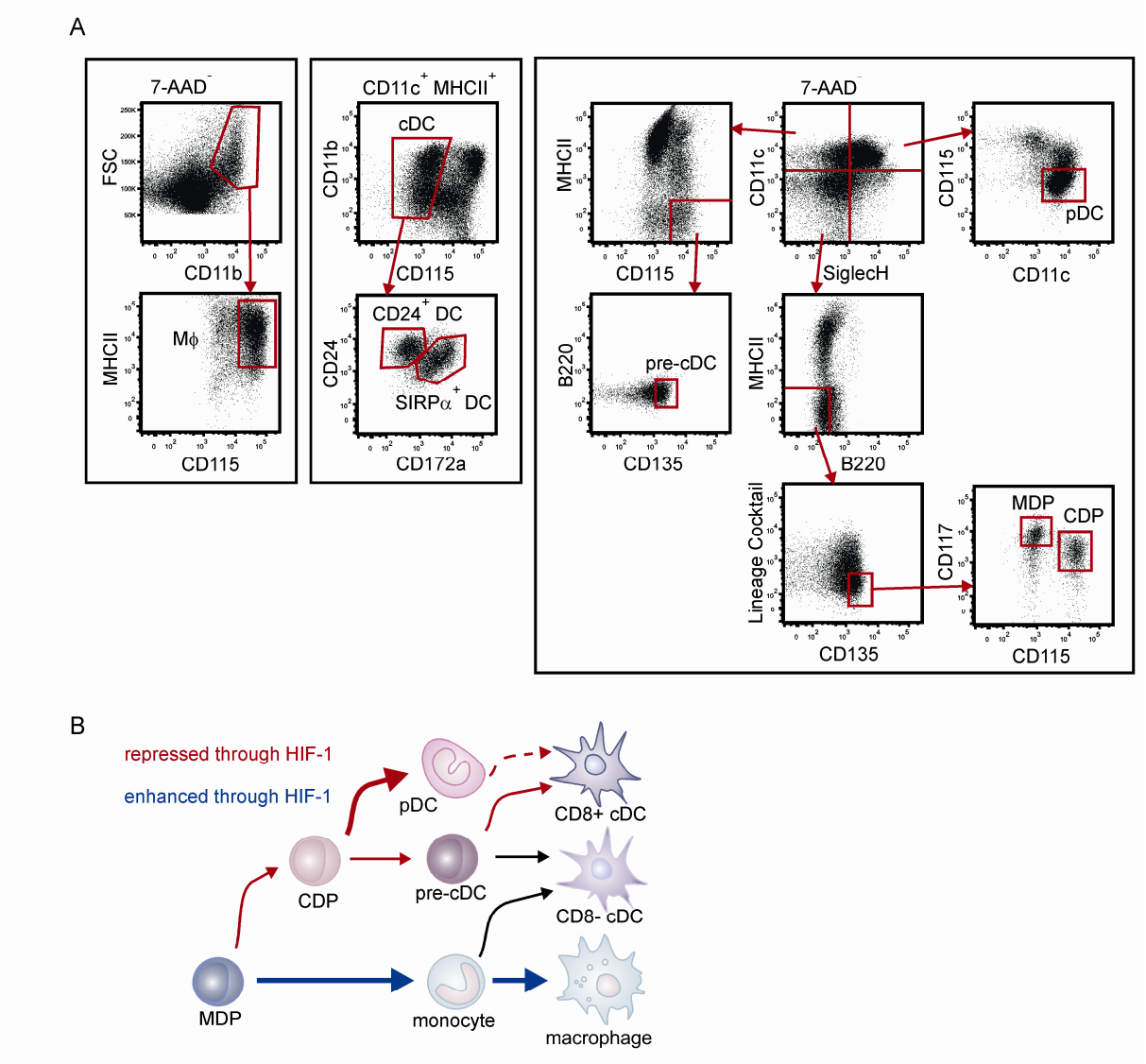

Figure S2. Gating strategy for different stages of mononuclear phagocyte development and HIF-1-dependent changes in Flt3-L BM cultures. (A) Total BM cells were cultured with Flt-3L, harvested at day 9 and analyzed by flow cytometry. Live cells (7AAD$^-$) were subgated to define macrophages (Mϕ: CD11b$^+$, FSC^{high}, CD115$^+$, MHCII$^+$), cDC subpopulations (CD24$^+$ DC: CD11c$^+$MHCII$^+$CD115$^-$ CD24^{high}CD172a$^-$ cDC; SIRPα$^+$ DC: CD11c$^+$MHCII$^+$CD115$^-$CD24$^{int/low}$CD172a$^+$), pDC (SiglecH$^+$CD11c$^+$CD115$^-$), pre-cDC (CD11c$^+$MHCII$^-$CD115$^+$CD135$^+$), common DC progenitors (CDP: Lin$^-$CD135$^+$CD117^{int}CD115$^+$) and monocyte/DC progenitors (MDP:

$Lin^{-}CD135^{+}CD117^{+}CD115^{low}$). The data shown are representative of at least three independent experiments with cells of 6-8 mice each, which produced similar results. The gating strategy is based on previously published data.[2-6] (B) The diagram shows the influence of HIF-1 expression on the development of the mononuclear phagocyte lineage, which was defined earlier[7].

Figure S3

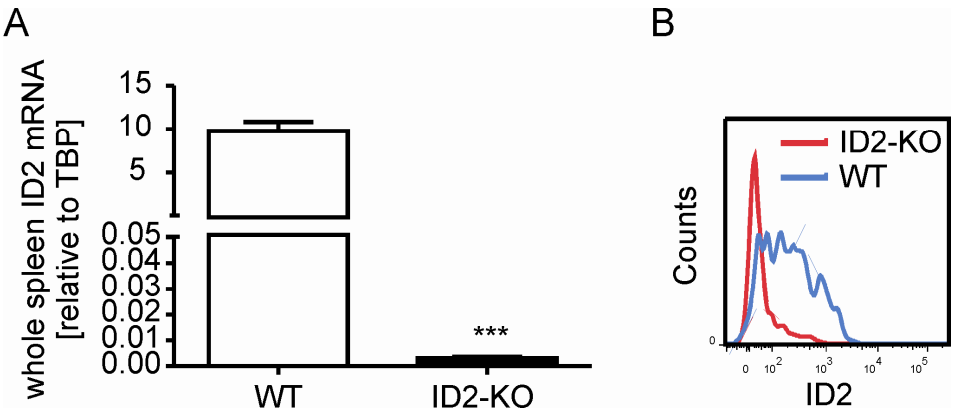

Figure S3. Validation of ID2 qPCR and intracellular staining using ID2-KO mice. (A) Relative ID2 mRNA expression in spleens of WT and ID2-KO mice quantified by qPCR is displayed. Data are means ± SEM of 3 animals of each genotype. (B) Intracellular expression of ID2 in isolated MDP/CDP of WT and ID2-KO mice upon 48h culture with 200 ng/ml Flt3-L was quantified by flow cytometry using biotin-coupled ID2 antibodies and streptavidin-PE-CF495. A representative histogram of 3 independent experiments using pooled cells of 2-3 mice each is shown.

Figure S4

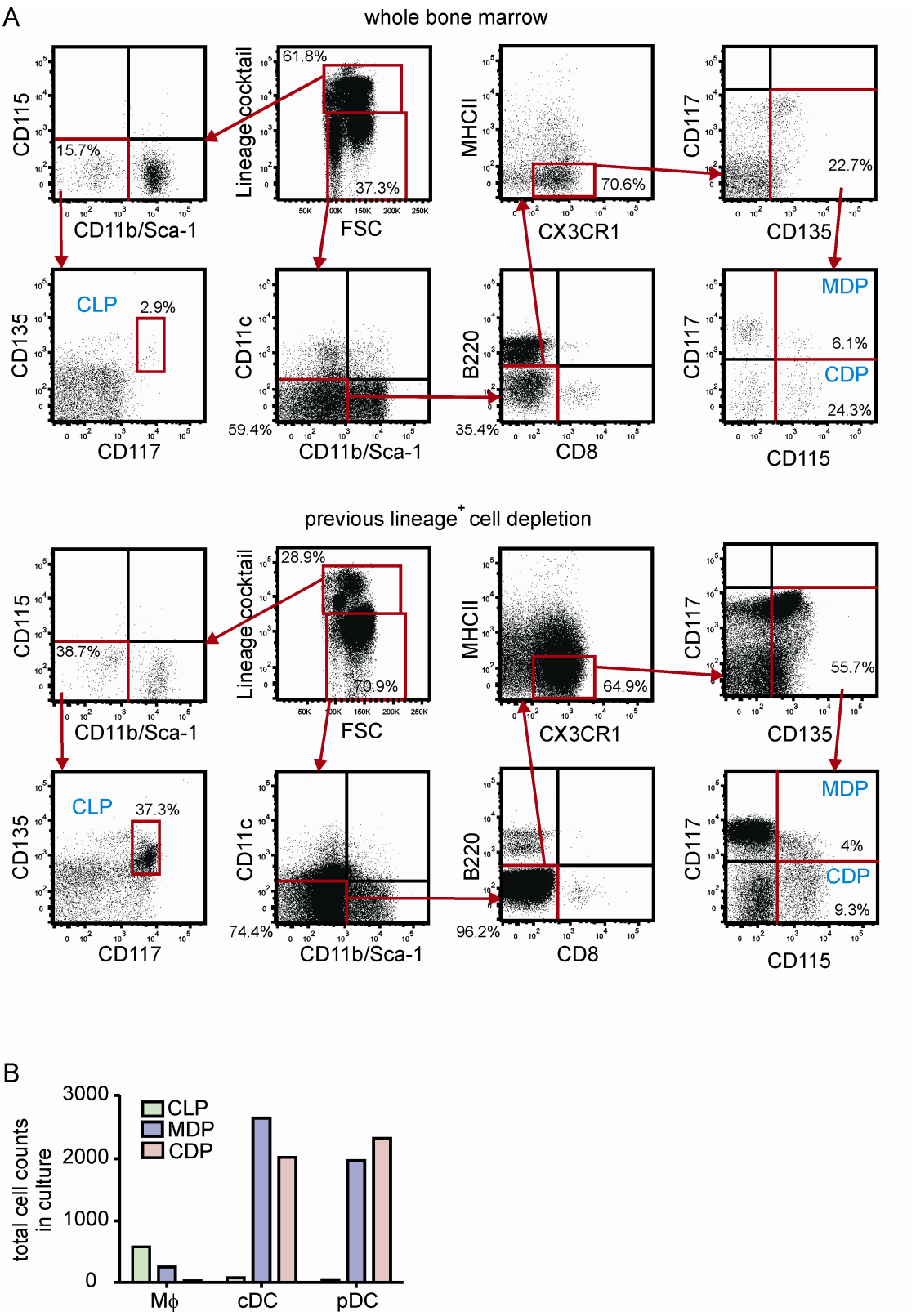
A
whole bone marrow
CD115
15.7%
CD11b/Sca-1
Lineage cocktail
61.8%
37.3%
FSC
MHCII
70.6%
CX3CR1
CD117
22.7%
CD135
CD135
CLP
2.9%
CD117
CD11c
59.4%
CD11b/Sca-1
B220
35.4%
CD8
CD117
MDP
6.1%
CDP
24.3%
CD115
previous lineage+ cell depletion
CD115
38.7%
CD11b/Sca-1
Lineage cocktail
28.9%
70.9%
FSC
MHCII
64.9%
CX3CR1
CD117
55.7%
CD135
CD135
CLP
37.3%
CD117
CD11c
74.4%
CD11b/Sca-1
B220
96.2%
CD8
CD117
MDP
4%
CDP
9.3%
CD115
B
total cell counts in culture
3000
2000
1000
0
CLP
MDP
CDP
Mϕ
cDC
pDC

Figure S4. Sorting of bone marrow progenitors and their DC differentiation potential *in vitro*. (A) Total BM was either enriched for lineage$^-$ cells using untouched magnetic separation (lower panel) or left untreated (upper panel) and common monocyte/DC progenitors (MDP), common DC progenitors (CDP) as well as common lymphocyte progenitor (CLP) were isolated using FACS sorting. The gating strategy for identification of MDP, CDP and CLP is displayed. MDP were defined as Lin$^-$CD11b$^-$Sca-1$^-$CD11c$^-$B220$^-$CD8$^-$MHCII$^-$CX3CR1$^+$CD135$^+$CD117$^+$CD115^{low} cells, CDP as CD11b$^-$Sca-1$^-$CD11c$^-$B220$^-$CD8$^-$MHCII$^-$CX3CR1$^+$CD135$^+$CD117^{low}CD115$^+$ cells and CLP as Lin$^+$(specifically CD127$^+$)CD115$^-$CD11b$^-$Sca1$^-$CD117^{high}CD135$^+$ cells. The data shown are representative of at least three independent experiments using 2-3 mice each, which produced similar results. Gating strategies are based on previously published data.[2-6] (B) 5000 MDP, CDP or CLP were cultured with 200 ng/ml Flt3-L in ultra-low attachment plates for 6 d and the generation of conventional dendritic cells (cDC), plasmacytoid dendritic cells (pDC) or macrophages (Mϕ) was analyzed by FACS as described in Figure S2. CLP served as the negative control. A representative experiment out of three is displayed.

Figure S5

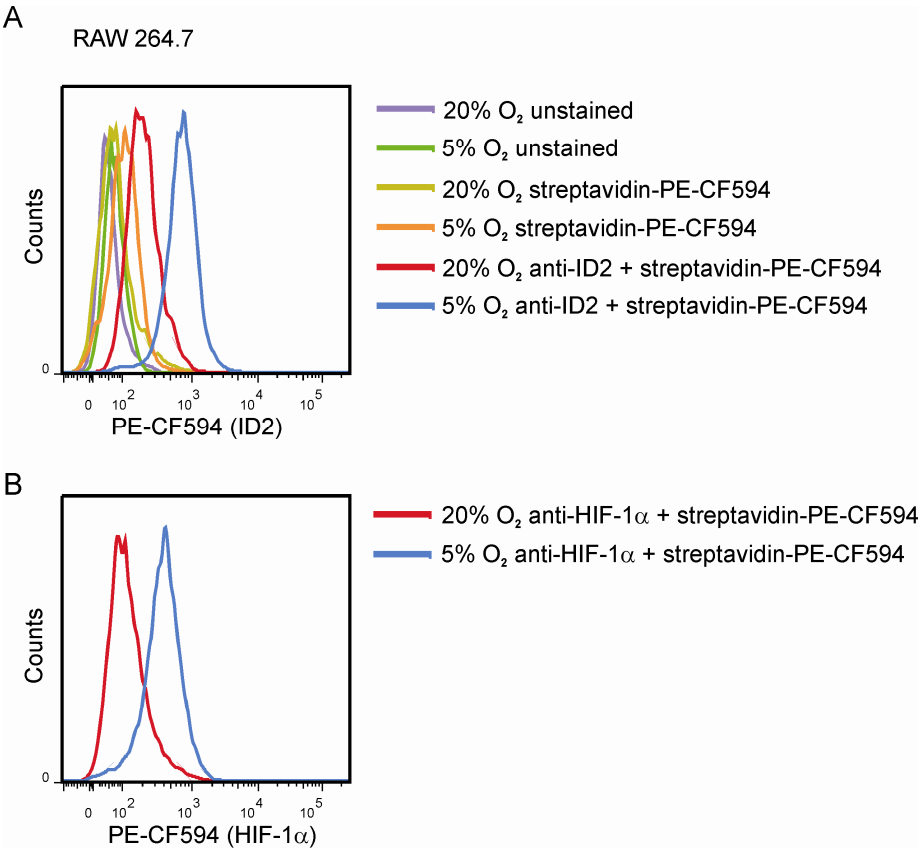

Figure S5. Establishing intracellular detection of ID2 and HIF-1α expression by flow cytometry. Intracellular expression of ID2 and HIF-1α in murine macrophage-like RAW264.7 cells cultured for 48 h at 20% or 5% O_2. Cells were harvested, fixed, permeabilized and blocked with 20 ng/ml unlabelled streptavidin to block endogenous biotin. Subsequently cells were left untreated or were incubated with PE-CF594-coupled streptavidin with or without addition of biotinylated anti-mouse-ID2 (A) or anti-mouse HIF-1α (B) antibodies. Samples were acquired by FACS. Data are representative of 3 independent experiments. Details of the staining procedure can be found under supplementary Methods.

Figure S6

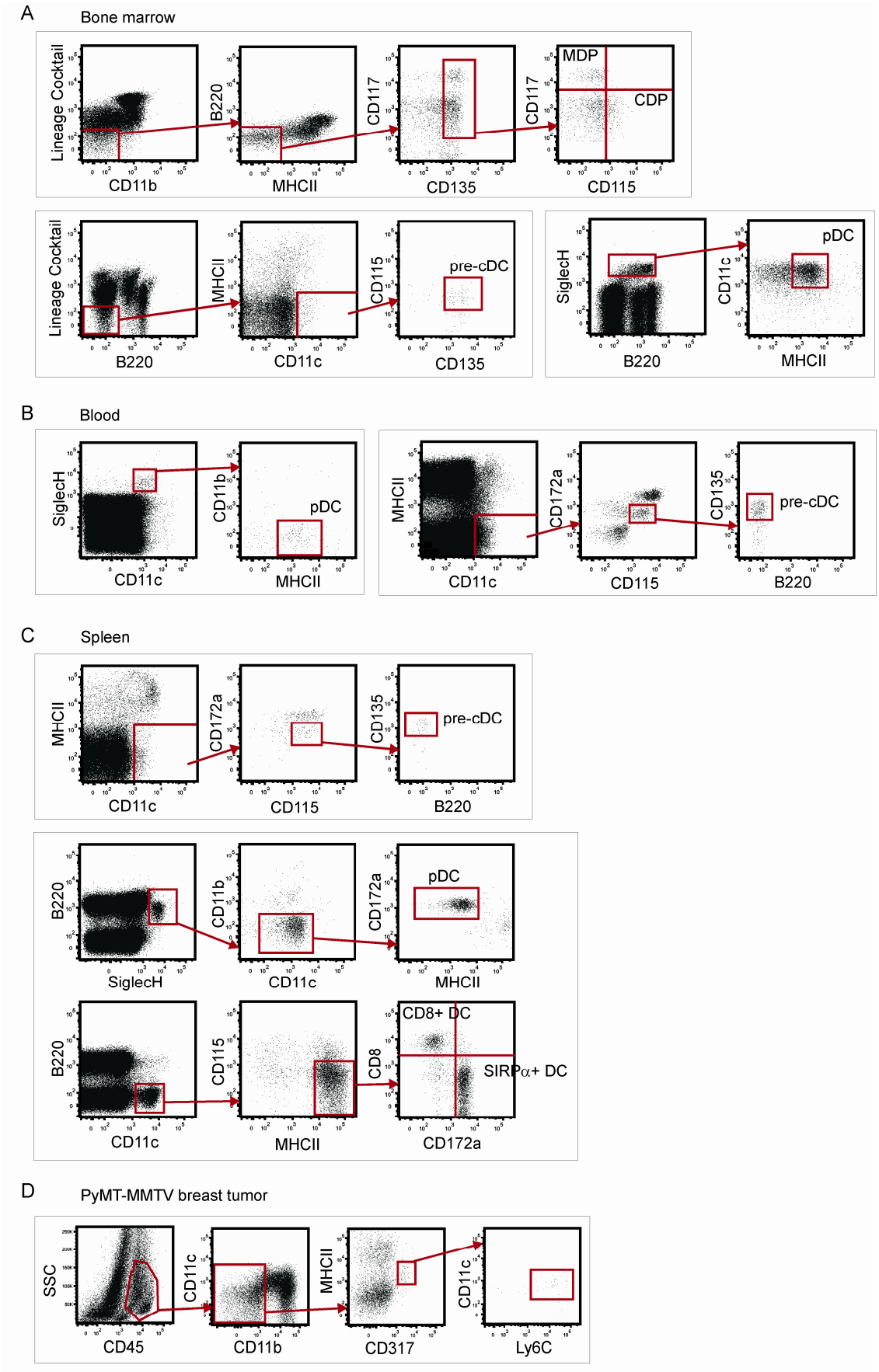
A
Bone marrow
Lineage Cocktail
CD11b
B220
MHCII
CD117
CD135
CD117
CD115
MDP
CDP
Lineage Cocktail
B220
MHCII
CD11c
CD115
CD135
pre-cDC
SiglecH
B220
CD11c
MHCII
pDC
B
Blood
SiglecH
CD11c
CD11b
MHCII
pDC
MHCII
CD11c
CD172a
CD115
CD135
B220
pre-cDC
C
Spleen
MHCII
CD11c
CD172a
CD115
CD135
B220
pre-cDC
B220
SiglecH
CD11b
CD11c
CD172a
MHCII
pDC
B220
CD11c
CD115
MHCII
CD8
CD172a
CD8+ DC
SIRPα+ DC
D
PyMT-MMTV breast tumor
SSC
CD45
CD11c
CD11b
MHCII
CD317
CD11c
Ly6C

Figure S6. Gating strategy for DC progenitors and DC subtypes in BM, blood, spleen and transgen-induced tumors. (A) Total BM was analyzed by flow cytometry. Live cells ($7AAD^-$) were subgated to define individual populations. MDP were defined as $Lin^-CD11b^-MHCII^-B220^-CD135^+CD117^+CD115^{low}$ cells, CDP as $Lin^-CD11b^-MHCII^-B220^-CD135^+CD117^{int}CD115^+$, pre-cDC as $Lin^-CD11c^+MHCII^-B220^-CD135^+CD115^+$ and pDC as $SiglecH^+B220^{+/int}CD11c^+MHCII^{int}$. (B) RBC-depleted whole blood was analyzed for pDC ($SiglecH^+CD11c^{int}MHCII^{int}CD11b^-$ cells) and pre-cDC ($CD11c^+MHCII^-CD115^+CD135^+CD172a^{low}$ cells). (C) Spleen single cell suspensions were analyzed for pre-cDC ($CD11c^+MHCII^-CD115^+CD135^+CD172a^{low}$ cells), pDC ($SiglecH^+B220^+CD11c^{int}CD11b^-MHCII^{int}CD172a^+$ cells) and cDC subsets ($CD8^+$ DC: $CD11c^+B220^-MHCII^+CD115^-CD8^+CD172a^-$; $SIRP\alpha^+$ DC: $CD11c^+B220^-MHCII^+CD115^-CD8^-CD172a^+$). (D) Single cell suspensions were created from mammary tumors of 100 day-old mice expressing the PyMT oncogene and analyzed by FACS. pDC were identified as $CD45^+CD11b^-CD11c^{int}HLA\text{-}DR^{int}CD317^+Ly6C^+$. All data shown are representative of at least three independent experiments using 2-8 mice each, which produced similar results. Gating strategies are based on previously published data.[2-6]

Figure S7

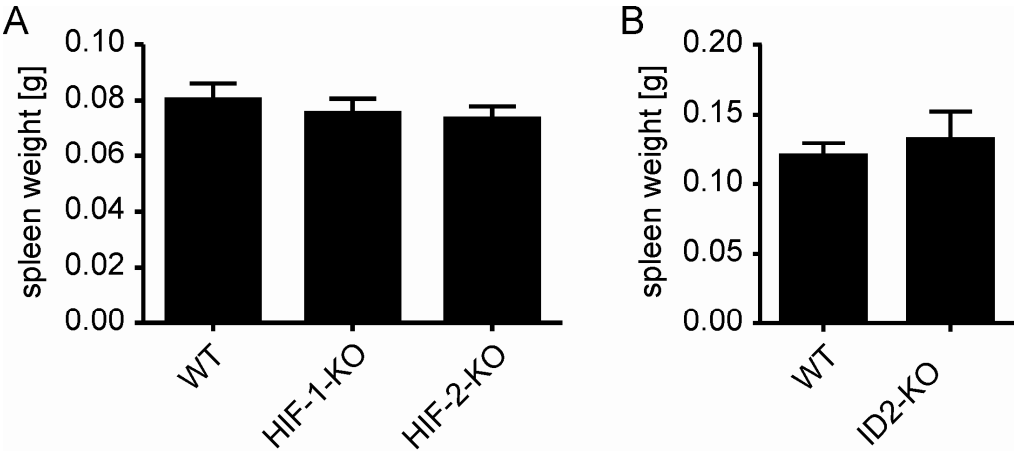

Figure S7. Spleen size of mouse strains employed in this study. Spleens were harvested from (A) C57BL/6 wildtype (WT), C57BL/6 HIF-1$\alpha^{fl/fl}$ LysM-Cre (HIF-1-KO), C57BL/6 HIF-2$\alpha^{fl/fl}$ LysM-Cre (HIF-2-KO) or (B) NMRI wildtype (WT), ID2$^{-/-}$ (ID2-KO) mice and the weight was determined. Data are means ± SEM from 6-10 individuals animals per genotype.

Supplementary References

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6. Zhang J, Raper A, Sugita N, et al. Characterization of Siglec-H as a novel endocytic receptor expressed on murine plasmacytoid dendritic cell precursors. *Blood.* 2006;107(9):3600-3608.
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