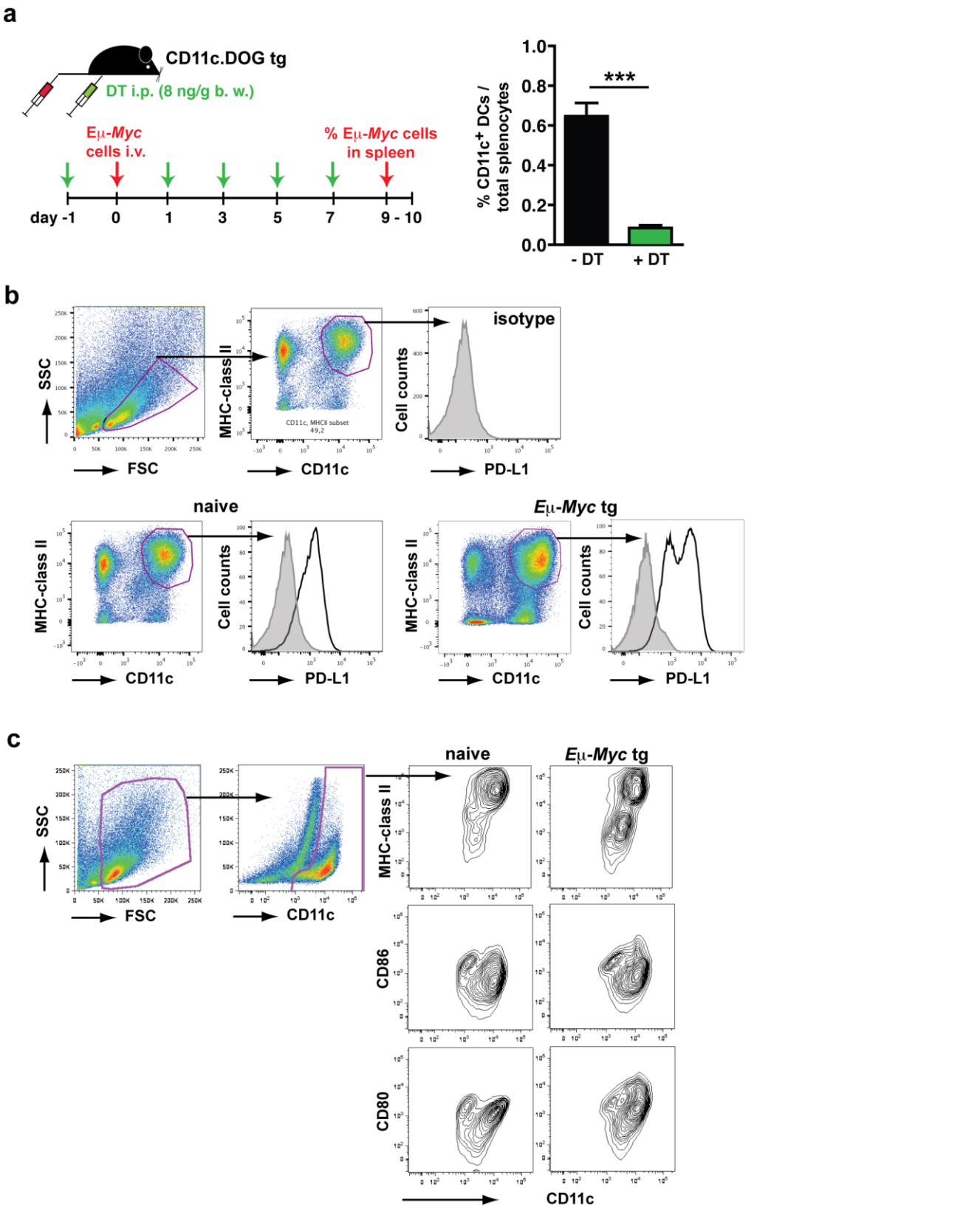
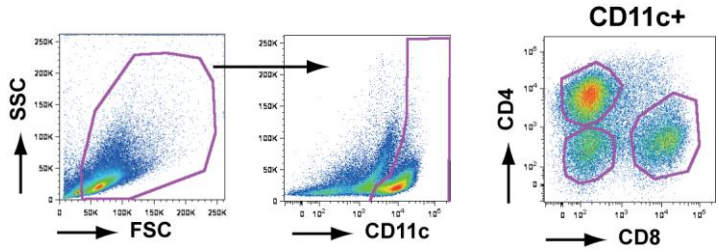

a
CD11c.DOG tg
DT i.p. (8 ng/g b. w.)
Eμ-Myc cells i.v.
% Eμ-Myc cells in spleen
day -1
0
1
3
5
7
9 - 10
% CD11c+ DCs / total splenocytes
1.0
0.8
0.6
0.4
0.2
0.0
***
- DT
+ DT
b
SSC
FSC
MHC-class II
CD11c
CD11c, MHCII subset
49,2
Cell counts
isotype
PD-L1
naive
Eμ-Myc tg
c
SSC
FSC
CD11c
naive
Eμ-Myc tg
MHC-class II
CD86
CD80
CD11c

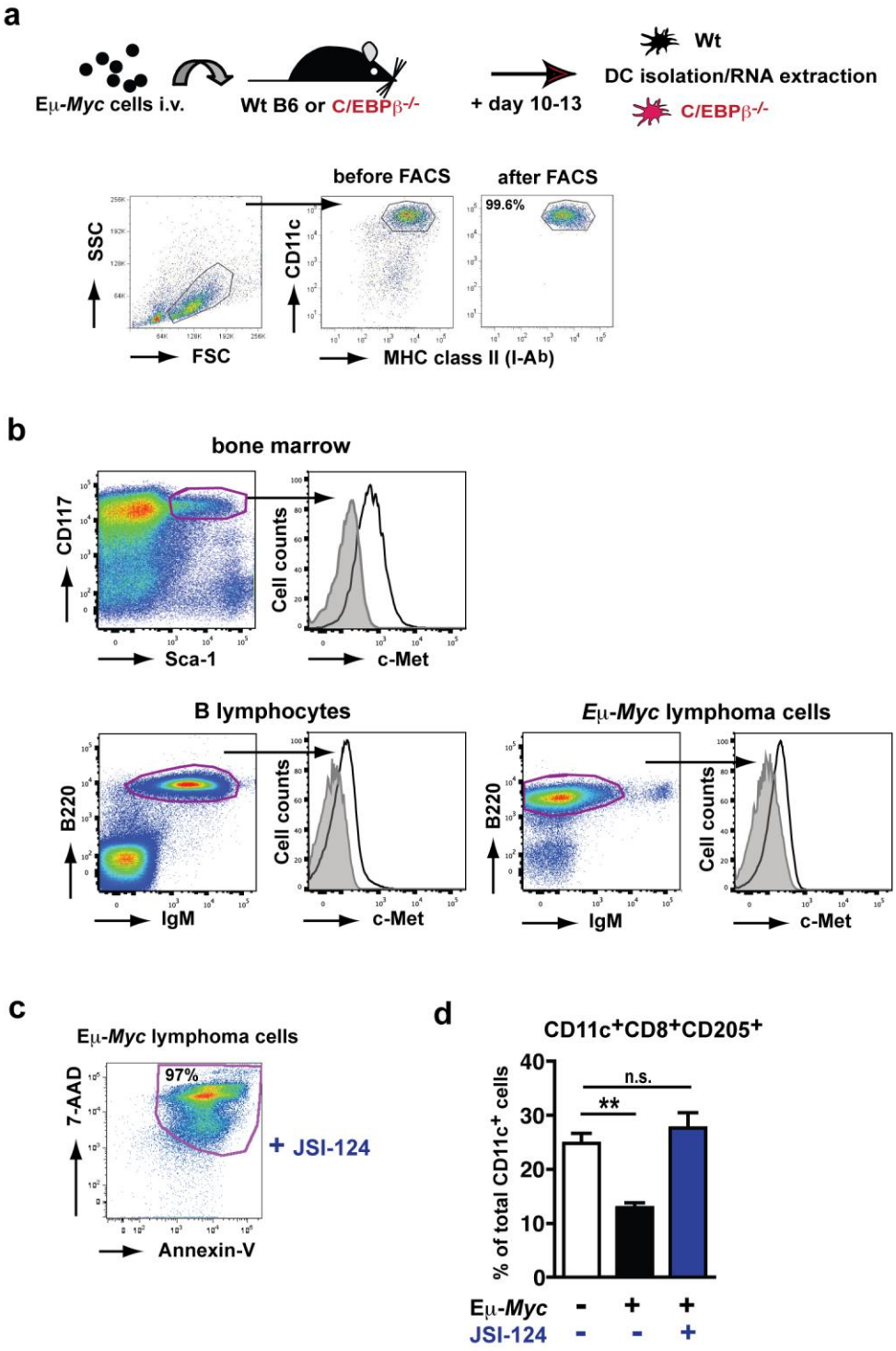
Supplementary Figure 1. Efficient DC depletion in CD11c.DOG transgenic mice

(**a**) CD11c.DOG transgenic mice (tg) were treated with 8 ng/g body weight (b.w.) diphtheria toxin (DT) i.p. on day -1 and every other day. The percentage of $CD11c^+$ DCs/total splenocytes in DT treated (green bars; n=24) compared to control mice (black bars; n=22) was assessed by flow cytometry 9-10 days after tumor challenge. Bars represent means ± s.e.m. ***P < 0.001. A Mann-Whitney test was used. (**b**) Spleens from Eμ-*Myc* transgenic mice (>3 months), or from naive B6 mice were injected with collagenase D containing buffer, followed by gentle disruption and incubation at 37°C for 30 min. DCs were analyzed by immunofluorescence staining with the antibodies indicated, dead cells were excluded by DAPI-staining. Shown is a representative example of the gating strategy for $CD11c^+$/MHC class II^+/$PD\text{-}L1^+$ DCs; shaded curve, isotype control. (**c**) Representative example for the gating strategy of all $CD11c^+$ DCs and contour plots to compare the distribution of MHC class II^{high} and MHC class II^{low} DCs in tumor-naive and Eμ-*Myc* transgenic mice. $CD11c^+$ cells were also costained fort the costimulatory molecules CD80 and CD86.



Supplementary Figure 2. Flow cytometry analysis of splenic DCs

Spleens from mice after transfer of Eμ-*Myc* lymphoma cells (day 10-13), or from untreated mice (day 0) were injected with collagenase D containing buffer, followed by gentle disruption and incubation at 37°C for 30 min. DCs were enriched with a CD11c MACS bead kit, followed by immunofluorescence staining with the antibodies indicated. Shown is a representative example of MACS-enriched $CD11c^+$ DCs (middle panel), and the application of subset specific secondary markers in the right panel. In some cases, MACS bead isolation was omitted and the frequency of $CD11c^+$ DCs was analyzed relative to all splenocytes (see Fig. 2). Gates in the dot blot on the right depict subsets among all $CD11c^+$ DCs.

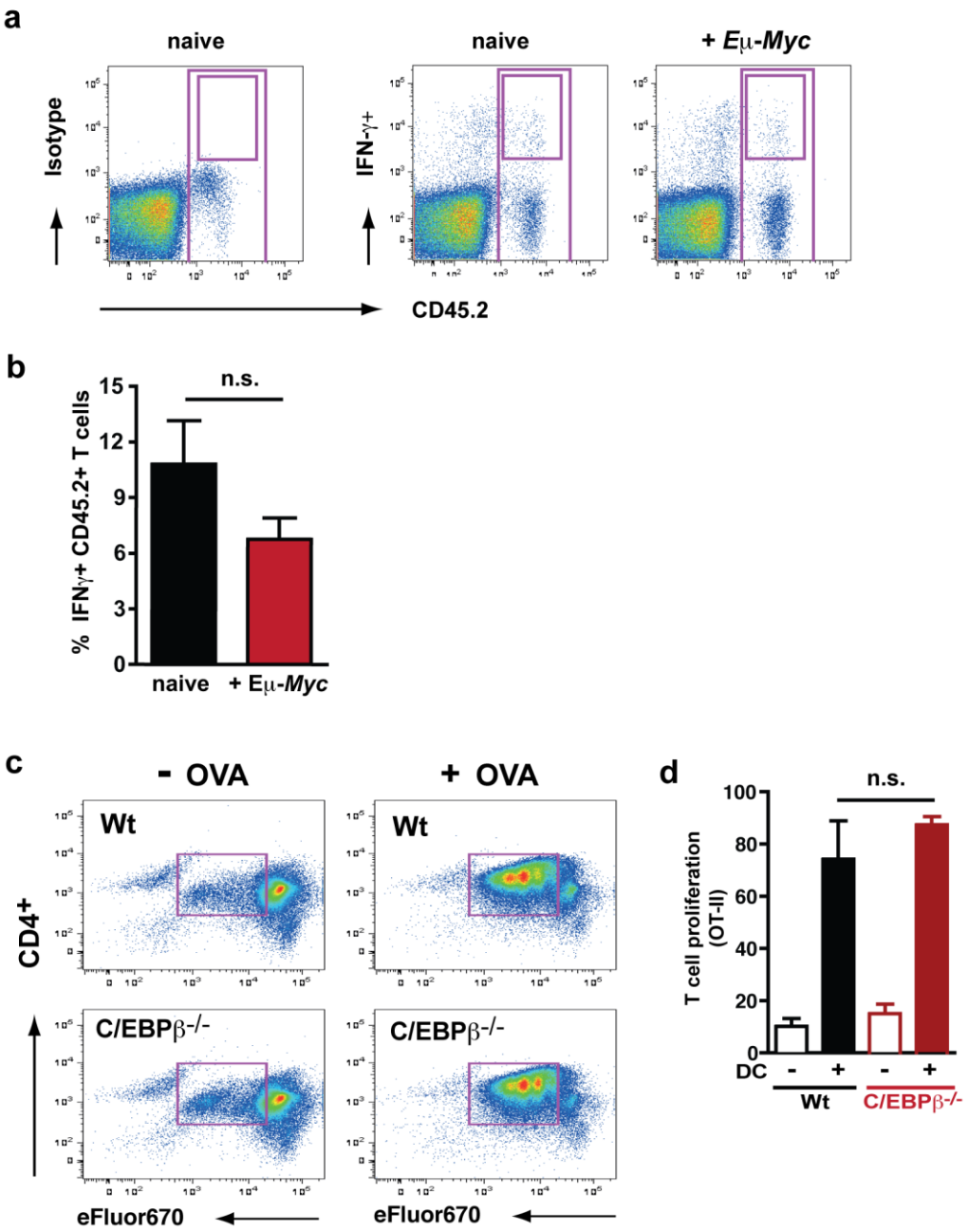


Supplementary Figure 3. Flow cytometry sorting of splenic DCs

(**a**) Isolation of splenic DCs from B6 or CD11c-Cre x C/EBPβ$^{-/-}$ (C/EBPβ$^{-/-}$) mice after transfer of Eμ-*Myc* lymphoma cells (day 10-13), or from untreated mice (day 0). Spleens were digested with collagenase D, followed by DC enrichment with CD11c

MACS beads and further purification by FACS sorting using CD11c and I-A^b antibodies. Shown are representative dot blots of $CD11c^+$ I-A^{b+} DCs prior and after FACS sorting. Number indicates percentage of double positive cells. (**b**) Flow cytometry analysis of the c-Met receptor expression in Sca-1^+/$CD117^+$ bone marrow hematopoietic stem cells (positive control), normal splenic B lymphocytes, and *Eμ-Myc* lymphoma B cells $B220^{high}$/IgM^{low} (n>3 animals). Shaded curve, isotype control.

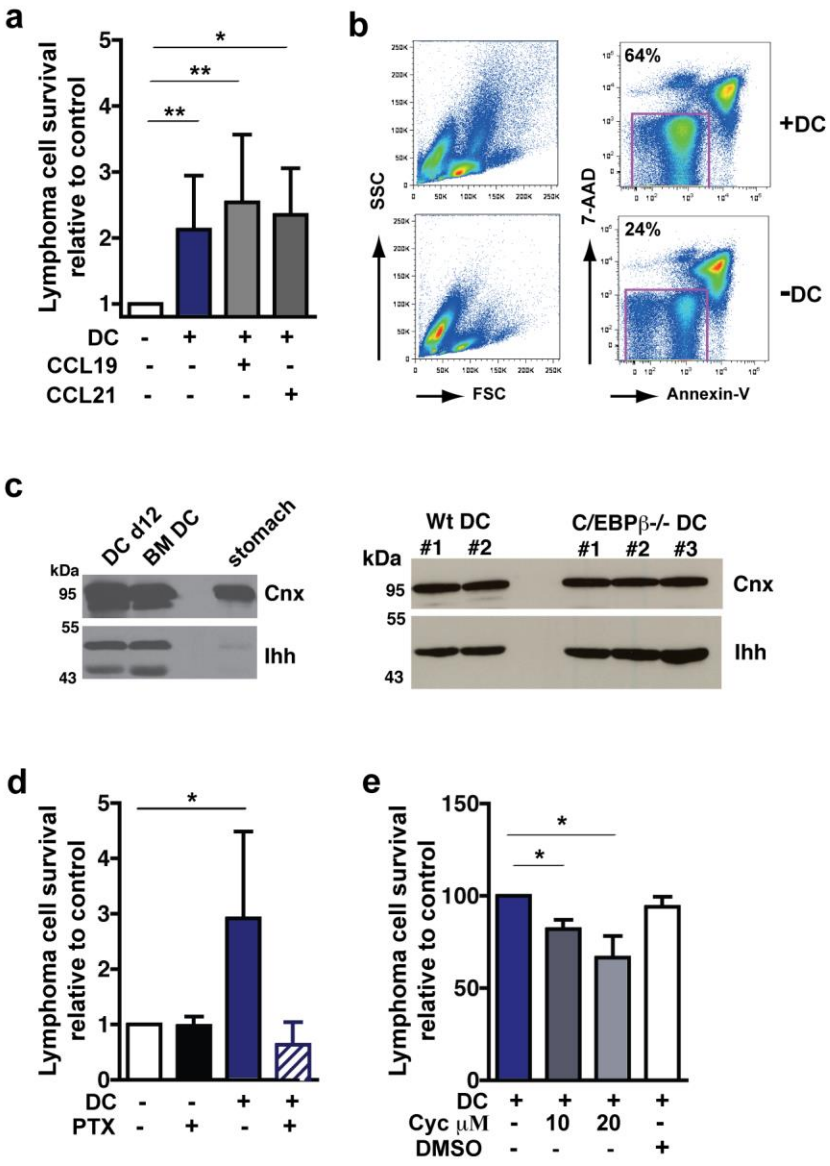
(c) *Eμ-Myc* lymphoma B cells were isolated from diseased mice (n=3) and cultured in the presence of JSI-124 at 0,5 ng/ml in lymphoma medium. After 24 hrs, tumor cells were gated for $B220^+$, and lymphoma cell viability was assessed by Annexin-V and 7-AAD staining. A representative dot plot shows the percentage of late apoptotic gated $B220^+$ Annexin-V^+ /7-AAD^+ cells. (**d**) Mice (B6) were adoptively transferred with *Eμ-Myc* lymphoma B cells (n=5) or left untreated (n=4). Starting at day 7, mice (n=3) were treated with the Stat3-inhibitor JSI-124 (Cucurbitacin I) at 1 mg/kg/b.w. per day (i.p.) for a total of 6 applications (day 7-14). Spleens were removed and analyzed for DC maturation and subset composition as in Fig. 2. A representative quantification for the JSI-124 induced effects on DC maturation in lymphoma challenged mice is shown for the $CD11c^+$/$CD8^+$/$CD205^+$ subset. Bars represent means ± s.e.m. **P < 0.01; n.s., non significant. A Mann-Whitney test was applied.



Supplementary Figure 4. C/EBPβ$^{-/-}$ DCs are functional in T cell stimulation

(**a**) *Eμ-Myc* lymphoma-challenged mice have a reduced capacity to induce effector T cell differentiation. Congenic mice (B6, CD45.1) were transplanted with *Eμ-Myc* lymphoma B cells (CD45.2). At day 7, OT-II CD4^{+} T cells (8 x 10^{5}) were adoptively transferred into tumor-naive or *Eμ-Myc*-challenged mice, and 20 hrs later OVA protein (100 μg) together with CPGs (25 μg; ODN 1668) were injected s.c. In these

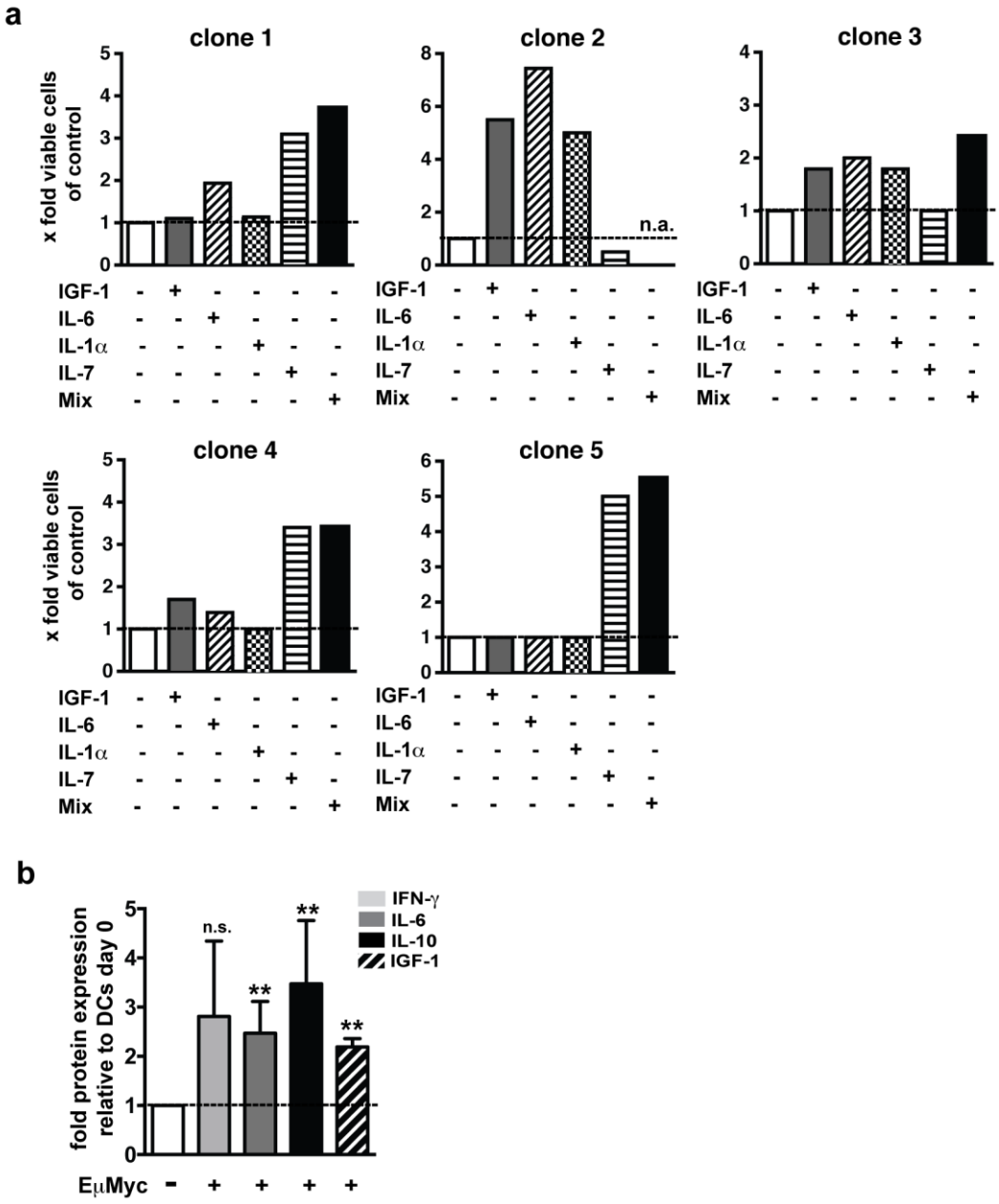
representative dot plots, $CD4^+$ gated lymph node cells were further gated for CD45.2 surface and intracellular IFN-γ staining. The inner gate indicates the frequency of the $CD4^+$ $CD45.2^+$ IFN-γ^+ subset. (**b**) Quantification of the percentages of OT-II effector $CD4^+$ IFN-γ^+ T cells in naive (n= 4) and *Eμ-Myc* lymphoma challenged mice (n=4). Bars represent means ± s.e.m.; n.s., non significant. (**c**) Myeloid DCs derived from Wt B6 and CD11c-Cre x C/EBPβ$^{-/-}$ mice (H-2^b haplotype) were pulsed with or without 2 μg/ml OVA-peptide. Fluorescent dye-labeled naïve OT-II T cells (1×10^5) were stimulated with pulsed or unpulsed DCs (1×10^4) for 3 days. Cells were analyzed by flow cytometry, and T cells were gated for the expression of CD4 and eFluor670. Proliferation of T cells was evaluated according to eFluor670 dilution. Gates indicate proliferated cells. (**d**) Quantification of OT-II proliferation. Bars indicate the percentage of proliferated $CD4^+$ cells within the gate (n=2 independent experiments with DCs from at least 5 animals per group). Bars represent means ± s.e.m.; n.s., non significant. In b) and d), a Mann-Whitney test was applied.



Supplementary Figure 5. BM-derived DCs support *Eμ-Myc* lymphoma cell survival

(**a**) Eμ-*Myc* tumor cells co-cultured with myeloid DCs alone or together with chemokines. Lymphoma cell survival relative to control cells (without DCs) was assessed by flow cytometry. Results are shown as x-fold cell survival relative to controls, set arbitrarily to 1 (n=4–9 separate Eμ-*Myc* cell clones tested in each group; n>5 independent experiments). (**b**) On the left, forward scatter (FSC) and side scatter (SSC) profiles of freshly isolated Eμ-*Myc* lymphoma cells co-cultured for 22-24 h with or without DCs is shown. On the right, tumor cells were gated for $B220^{+}$, and

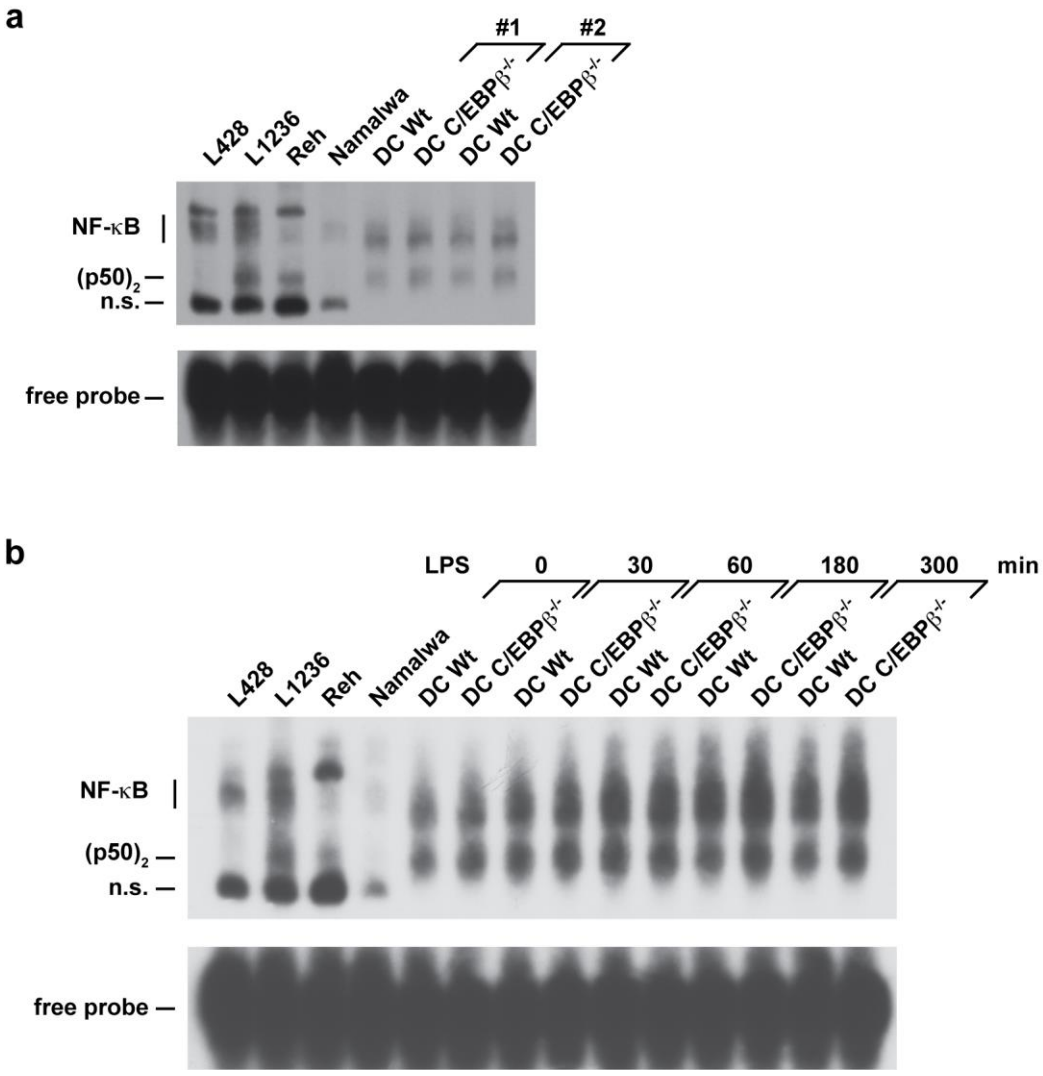
lymphoma cell viability was assessed by Annexin-V and 7-AAD staining. Representative dot plots and numbers show viable gated $B220^{+}$ Annexin-V^{-} /7-AAD^{-} cells. (**c)** Bone marrow (BM)–derived DCs release the hedgehog-pathway ligand indian hedgehog (Ihh). Left panel, Western blot detection of Ihh in DCs from tumor-bearing mice, and BM DCs (n>4 mice per group); Cnx, Calnexin. On the right, detection of Ihh in Wt (n=2) and in C/EBP$\beta^{-/-}$ DCs (n=3 different preparations of BM-derived DCs, with 2-3 animals pooled per preparation). (**d**) Eμ-*Myc* tumor cells co-cultured with myeloid DCs alone, or together with pertussis toxin (PTX). Lymphoma cell survival relative to control cells (without DCs) was assessed as in (b). (**e**) Viability of lymphoma cells grown on DCs with cyclopamine for 20–22 h. Results are shown as percent survival relative to control, set arbitrarily at 100% (n=3-4 independent experiments in (d, e). Bars represent means±s.e.m. *P<0.05; **P<0.01. In a), d) and e) a Student's t test was applied.



Supplementary Figure 6. Effect of cytokines on *Eμ-Myc* lymphoma B cell viability

NIH3T3 cells were plated and irradiated 4 hr later. Cells were grown overnight, and then freshly isolated *Eμ-Myc* lymphoma B cells (n=5 independent clones) were added; co-cultures were supplemented with the cytokines indicated. After 24 hours, tumor cells were gated for $B220^+$, and lymphoma cell viability was assessed by

Annexin-V and 7-AAD staining. Lymphoma cell survival relative to control cells (without cytokines) was assessed by flow cytometry. Results are shown as x-fold cell survival relative to controls, set arbitrarily to 1. **(b)** Splenic DCs from Wt naïve (n=6) and tumor-challenged mice (n=5) at day 10-13 were isolated by $CD11c^+$ MACS beads, followed by cell culture for 4 hr in the presence of PMA/ionomycin. Cell-free supernatant was analyzed by cytokine bead arrays for cytokine content. IGF-1 was determined by ELISA, DCs were from n=2 naive and n=4 lymphoma challenged mice. Cytokines upregulated more than 2-fold as compared to DCs from naïve mice are indicated as bars with means±s.e.m. **P<0.01; n.s., non-significant. A Mann-Whitney test was applied.

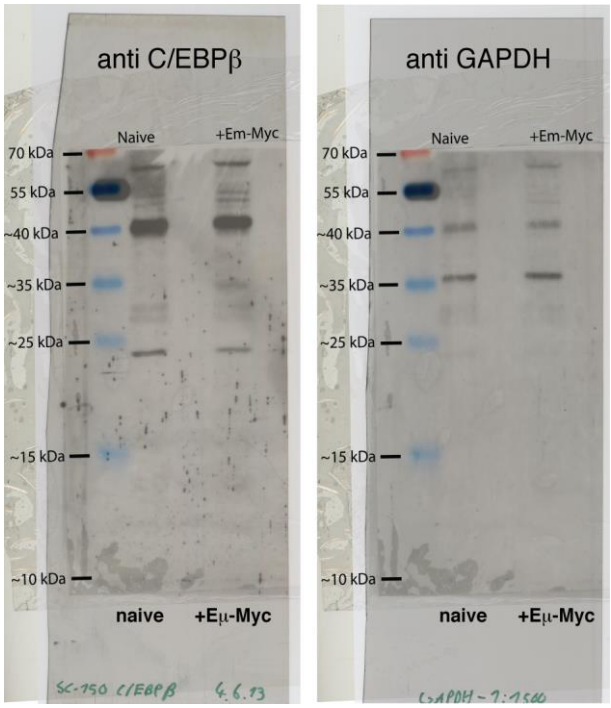


Supplementary Figure 7. DNA binding activity of NF-κB does not depend on C/EBPβ

(**a**) Analysis of NF-κB DNA-binding activity by electrophoretic mobility shift assay (EMSA) in unstimulated Wt and C/EBPβ$^{-/-}$ DCs. Whole cell extracts of the human cell lines L428, L1236, Reh and Namalwa as well as BM-derived murine Wt (B6) or C/EBPβ$^{-/-}$ DCs were analyzed by EMSA. Positions of canonical NF-κB (marked as NF-κB) and p50-homodimeric complexes [$(p50)_2$] are indicated; n.s., non-specific complex. In contrast to Reh and Namalwa cell lines, the Hodgkin lymphoma cell lines L428 and L1236 are characterized by constitutively active NF-κB activity. Notably, the non-specific complex is detectable in human cell lines but not in primary murine

cells. The human lymphoma and leukemia cell lines served as controls. DCs from two (#1 and #2) out of four independent experiments are shown.

(**b**) Analysis of NF-κB DNA-binding activity by EMSA in lipopolysaccharide (LPS) stimulated Wt and C/EBPβ$^{-/-}$ DCs. The assay was performed as in (a). DCs were treated for the indicated times with 1 μg/ml LPS. One experiment out of two independent experiments performed is shown.



Supplementary Figure 8. Uncropped images of a blot presented in the main paper.

This immunoblot refers to Figure 7a. Molecular weight marker is indicated in kDa. Naive, refers to DCs from tumor naive mice; +Eμ-Myc, refers to DCs isolated from Eμ-Myc lymphoma B cell-challenged mice.

Supplementary Methods

Antibodies

The following primary antibodies were used for flow cytometry: FITC and APC-labelled rat anti-mouse CD117; PE-labelled rat anti-mouse Sca-1 (Ly-6A/E); ; FITC and APC labelled rat anti-mouse IgM (all from Biolegend) For intracellular IFN-γ detection, an Alexa Fluor 647-labelled rat anti-mouse IFN-γ antibody was used (BD Biosciences. Antibodies were used in a 1:100 dilution.

Antigen-specific T cell proliferation assay *in vitro*

Bone marrow–derived DCs were generated as described in the main methods section . Quality and maturation of the DC preparations from Wt and *CD11c-Cre x C/EBPβ*$^{flox/flox}$ mice was assessed in flow cytometry by determining their forward and side scatter (FSC and SSC) morphology, in addition cells were stained with anti CD11c, CD86, and MHC class II antibodies. For each in vitro proliferation experiment, DCs were matched according to these differentiation markers. DCs were pulsed for 2-3 hrs with 2 µg/ml ovalbumin (OVA)-derived peptide aa323-339. Responder T cells from TCR transgenic OT-II mice (H-2^{b}) were isolated from splenocytes by negative selection using CD4 MACS beads (Miltenyi Biotec) according to the manufacturer's instructions, followed by eFluor® 670 cell labeling (eBioscience). Purity of CD4^{+} clonotypic T cells was always in the range >85%. 1 x 10^{5} eFluor-labeled T cells were mixed with 1x10^{4} unpulsed or pulsed DCs in round-bottom, 96-well tissue culture plates for three days. Cells were analyzed for fluorescent-dye dilution by flow cytometry.

Cytokine stimulation assay

NIH3T3 cells were seeded at 2.5 x 10^5 cells per well of a 6-well plate. To inhibit their proliferation the cell layer was irradiated 4 hr later (30 Gy). Cells were grown overnight, medium was exhanged and freshly isolated *Eμ-Myc* lymphoma B cells at 2 x 10^6/well (n=5 independent clones) were added; cocultures were supplemented with the cytokines indicated (IGF-1=100 ng/ml; IL-6=20 ng/ml; IL-1α=10 ng/ml; IL-7=50 U/ml), or a mix of all cytokines. After 24 hrs, tumor cells were gated for $B220^+$, and lymphoma cell viability was assessed by Annexin-V and 7-AAD staining. Lymphoma cell survival relative to control cells (without cytokines) was assessed by flow cytometry. Cytokines were from Peprotech or R&D, respectively. Cytokine concentrations were according to published reports or according to the manufacturer's instructions.

Determination of antigen-specific effector T cells *in vivo*

To determine the effector T cell frequencies in adoptively transferred mice, $CD4^+$ T cells from OT-II mice were adoptively transferred into CD45.1 congenic recipient mice, followed by OVA protein and CpG application the day thereafter. Three days later, single cell suspensions of inguinal lymph node cells were restimulated for 4 hrs with PMA (40 ng ml^{-1}) and ionomycin (1,5 μg ml^{-1}) in the presence of Brefeldin A (20 μg/ml); OT-II T cells were co-stained with anti CD4 anti CD45.2, followed by intracellular IFN-γ staining. Briefly, restimulated cells were washed and blocked for 15 min with anti-CD16/CD32. Subsequently, cells were stained with anti-CD4 and anti-CD45.2 for 30 minutes on ice. Cells were fixed, washed, and permeabilized with a Cytofix and Cytoperm kit (Biolegend), followed by blockade with anti-CD16/ CD32 antibody. After 15 min, anti-IFN-γ or isotype control antibody were added.

Data were acquired on a FACSCantoII flow cytometer (Becton Dickinson) and were further analyzed with FlowJo software (TreeStar).

Electrophoretic mobility shift assay (EMSA)

For whole cell extract preparation, cells were washed and resuspended in cell lysis buffer containing 20 mM HEPES pH 7.9, 350 mM NaCl, 1 mM $MgCl_2$, 0.5 mM EDTA, 0.1 mM EGTA, 1% (v/v) Nonidet-P40, 1 mM NaF, 10 mM Na_3VO_4, complete Mini protease inhibitor cocktail (Roche), and 1 mM DTT. Following a 10 min incubation on ice, the lysate was centrifuged for 5 min at 14 000 r.p.m. in a microfuge and the supernatant was used as whole cell extract. EMSA assays using (α-^{32}P)dCTP-labeled oligonucleotides were performed as described[1] using the following oligonucleotides: NF-κB sense 5´-AGCTCAGGGCTGGGGATTCCCCATCTCCACAGG, and NF-κB antisense 5´-AGCTCCTGTGGAGATGGGGAATCCCCAGCCCTG.

Supplementary Reference

1. Mathas, S. et al. Aberrantly expressed c-Jun and JunB are a hallmark of Hodgkin lymphoma cells, stimulate proliferation and synergize with NF-kappa B. *EMBO J* **21**, 4104-4113 (2002).