

Supplementary Materials for

Epigenetic stabilization of DC and DC precursor classical activation by TNF α contributes to protective T cell polarization

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The PDF file includes:

- Fig. S1. TNF α depletion in CBA/J mice results in fungal persistence and dysregulated immunity during *C. neo* infection.
- Fig. S2. Schematic of BMDC experiments and testing model.
- Fig. S3. TNFR1 and TNFR2 are both required for optimal DC1 programming.
- Fig. S4. TNF α results in sustained DC1 profile at the protein level in pulmonary DCs during *C. neo* infection.
- Fig. S5. MLL1 is uniquely induced in TNF α -programmed DC1.
- Fig. S6. DC1 from control *C. neo*-infected mice epigenetically resemble TNF α -programmed DC1.
- Fig. S7. Assessment of total and myeloid precursor populations in the BM during *C. neo* infection.

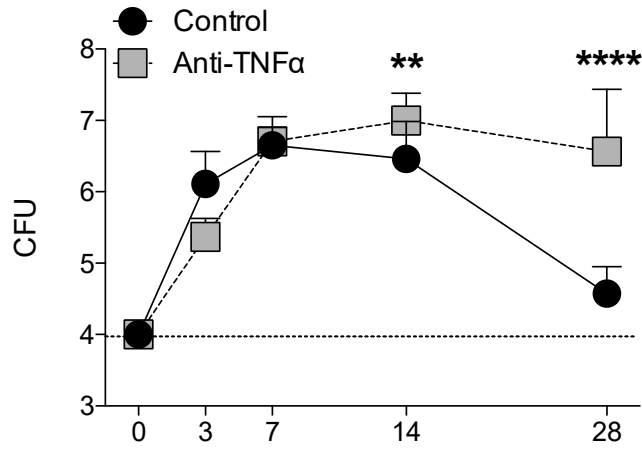
Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/5/12/eaaw9051/DC1)

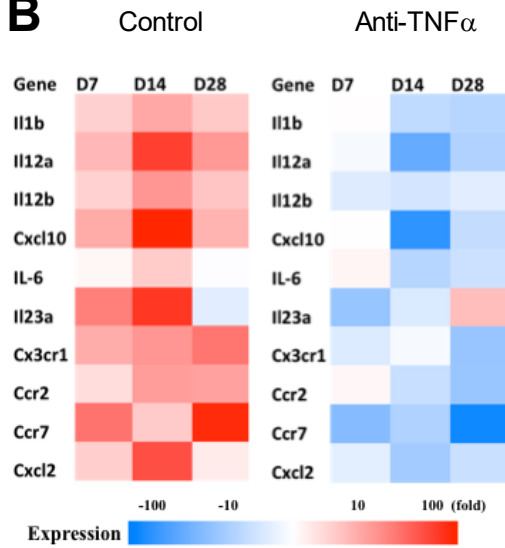
- Supplemental file 1. C.neo H3K4me3 Peaks.xlsx
- Supplemental file 2. 52D Infected Unique and Shared Peaks.xlsx
- Supplemental file 3. 52D Infected Gene Ontology.xlsx

Figure S1

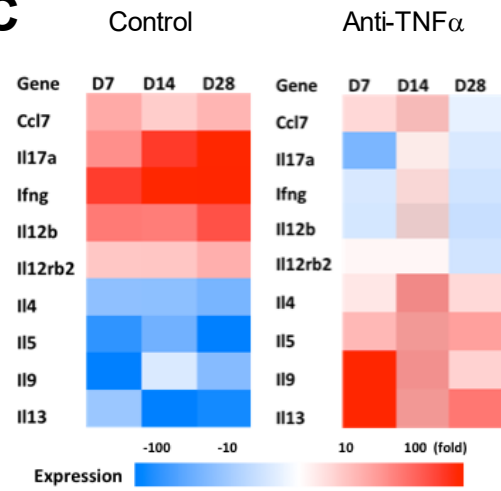
A



B



C



D

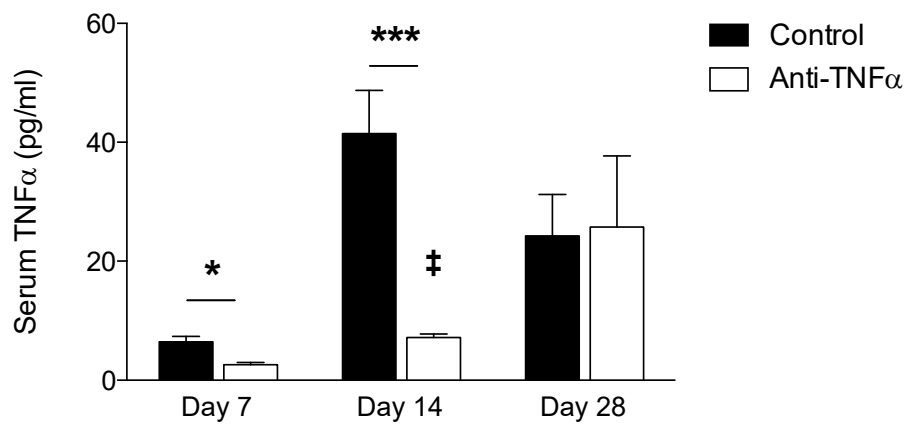
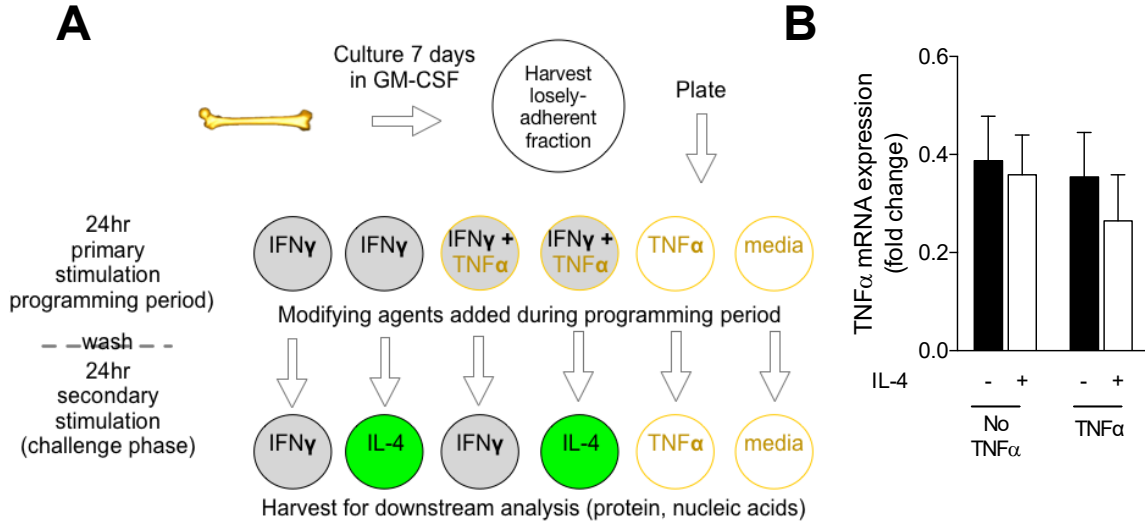
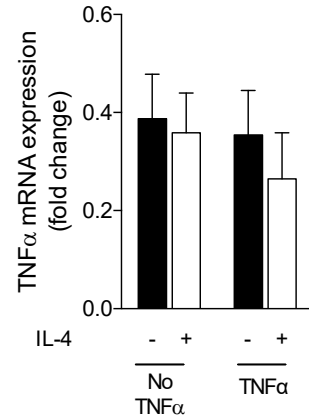


Fig. S1. TNF α depletion in CBA/J mice results in fungal persistence and dysregulated immunity during *C. neo* infection. (A) Pulmonary fungal burden was evaluated in *C. neo*-infected control and TNF α -depleted mice (anti-TNF α) at 3, 7, 14, and 28 dpi. (B) Magnetically sorted CD11c⁺ cells isolated from mouse lung at 7, 14, and 28 dpi. CT values are presented as a heatmap of gene expression in isotype-treated infected mice (left) and TNF α infected (right) mice relative to isotype-treated uninfected mice. N = 4-7 from two separate, matched experiments. (C) Magnetically sorted CD4⁺ cells isolated from mouse lung at 7, 14, and 28 dpi. CT values are presented as a heatmap of gene expression in isotype-treated infected mice (left) and TNF α infected mice (right) relative to isotype-treated uninfected mice. N = 4-7 from separate, matched experiments. (D) Cytometric bead array for TNF α was performed on serum from whole blood of infected mice. ‡ statistically significant difference between TNF α serum levels in α TNF α mice at 14 dpi compared to 7 dpi. N = 26-32 from five separate, matched experiments.

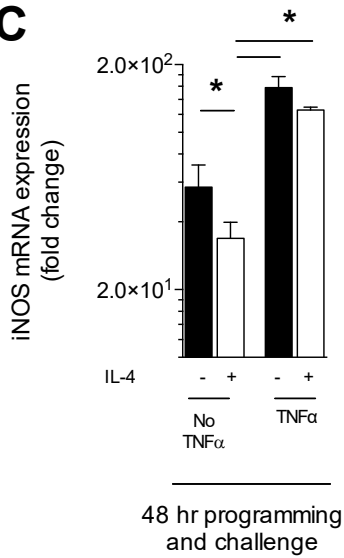
Figure S2



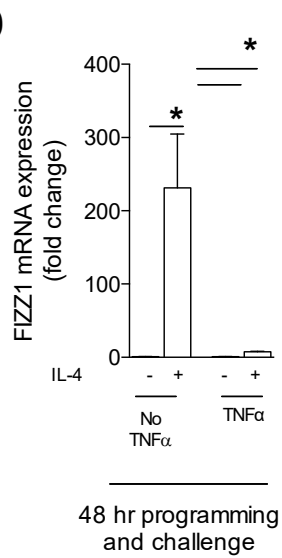
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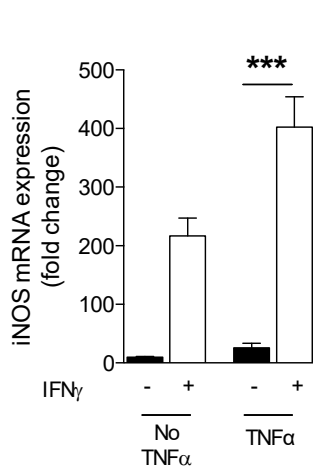
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D



E



F

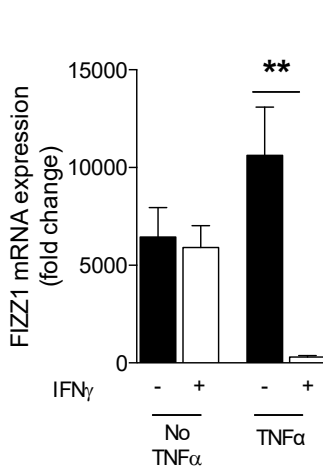


Fig. S2. Schematic of BMDC experiments and testing model. (A) BMDCs were treated for 24 hours with IFN γ in the presence or absence of TNF α , then washed and challenged with IL-4. Agents are added to disrupt the programming period during the first 24 hour incubation. Controls for all experiments include media only and TNF α only. (B) BMDCs were treated as according to A and did not induce TNF α mRNA transcript. N = 9 from three separate, matched experiments. (C-D) BMDCs were treated for 48 hours during the programming and challenge phases with similar results to 24 hour treatments. N=6 from two separate, matched experiments. * p < 0.05. (E-F) DC2 BMDCs polarized by IL-4 in the presence or absence of TNF α during the programming phase were challenged with IFN γ to determine whether TNF/ α -mediated programming is specific for DC1 or also applicable to DC2. iNOS (E) and Fizz1 (F) levels were analyzed by RT-qPCR. ** p < 0.01; *** p < 0.001

Figure S3

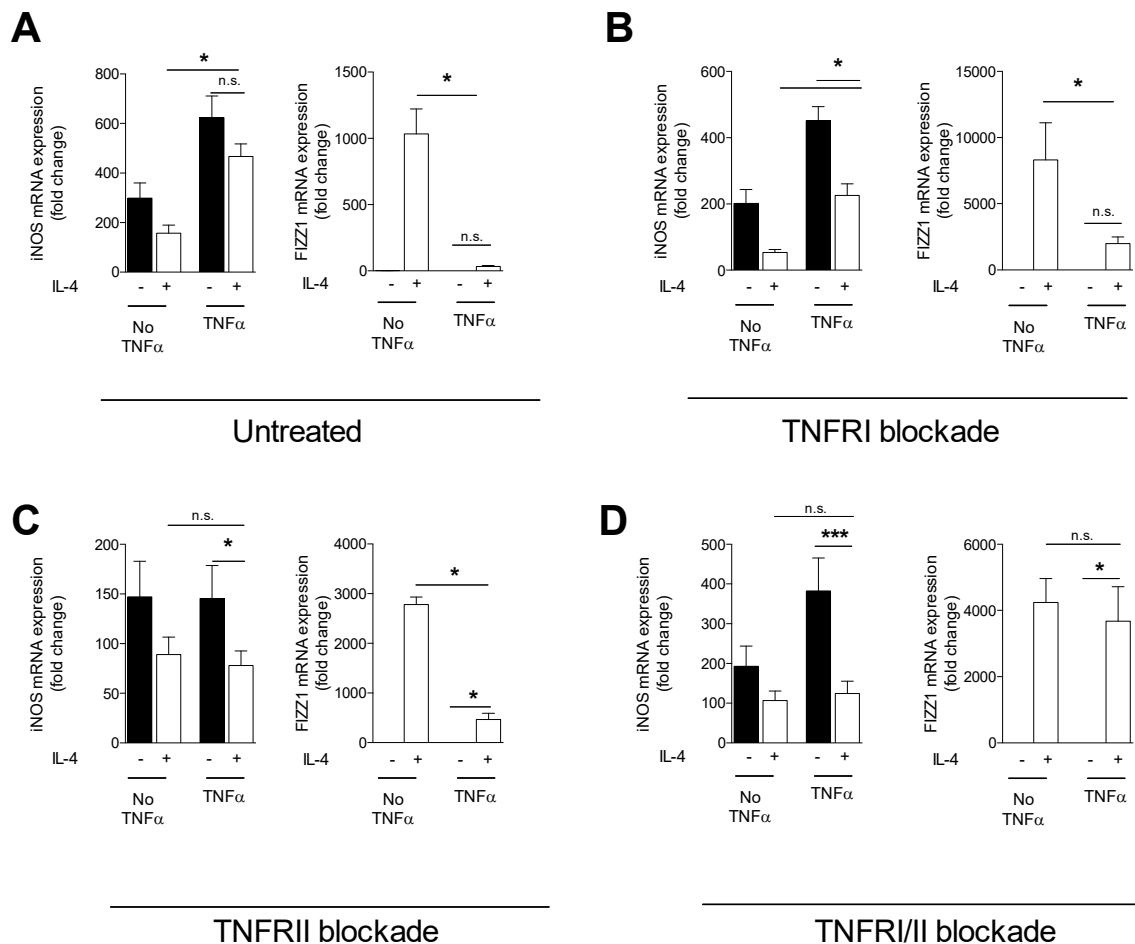


Fig. S3. TNFRI and TNFRII are both required for optimal DC1 programming.

BMDCs were treated for 24 hours with IFN γ in the presence or absence of TNF α (A) and one (B-C) or both (D) blocking antibodies for TNFRI and TNFRII, then washed and challenged with IL-4. Restoration of plasticity, both by expression of DC2 markers and suppression of DC1 markers upon IL-4 challenge in TNF α -programmed DCs, was assessed by evaluation of iNOS and Fizz1 mRNA expression. N = 6 from two separate, matched experiments. * p < 0.05. ** p < 0.01; *** p < 0.001; n.s. represents not significant.

Figure S4

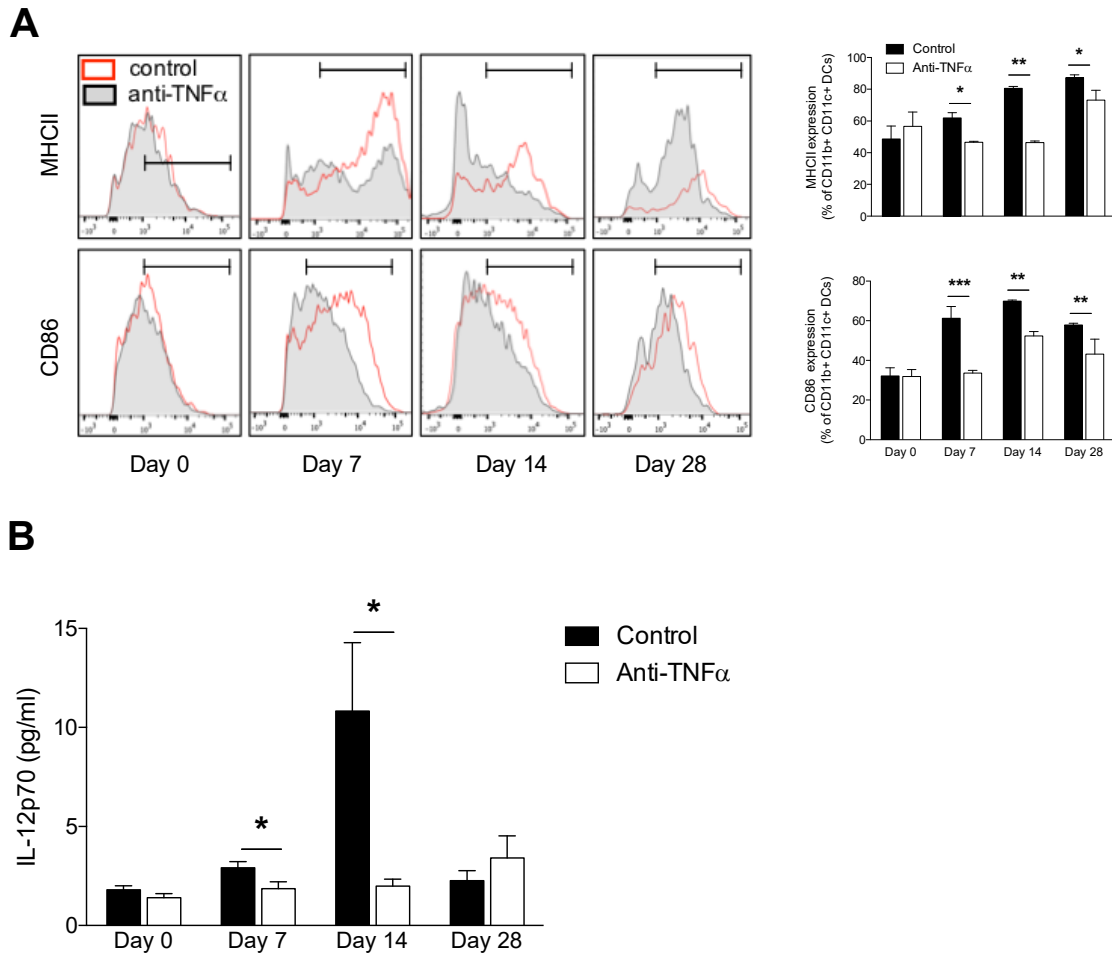


Fig. S4. TNF α results in sustained DC1 profile at the protein level in

pulmonary DCs during *C. neo* infection. (A) Extracellular flow cytometry for MHCII

and CD86 performed on enzymatically-digested lung from uninfected mice and at 7, 14,

and 28 dpi. DCs were gated on CD45⁺/CD19⁻/CD3⁻/Ly6G⁻/CD11b⁺/CD11c⁺.

Representative histogram overlays are presented with bar graphs representing average

percent positive. N = 8 from two separate, matched experiments. **(B)** Serum levels of IL-

12p70 from infected mice at 7, 14, and 28 dpi were assessed by cytometric bead assay.

N = 26-32 from five separate, matched experiments. * p < 0.05; ** p < 0.01; *** p < 0.001

Figure S5

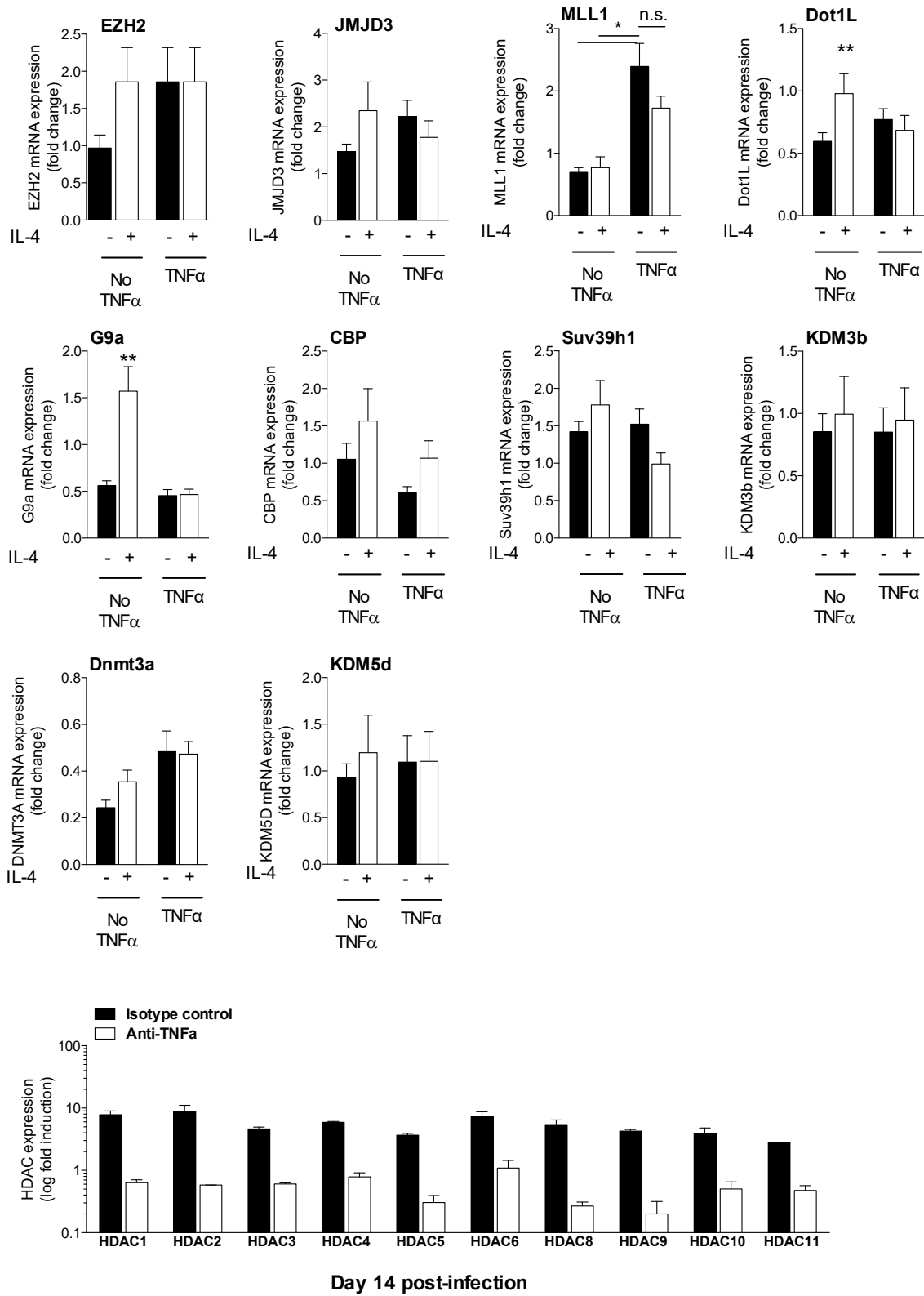


Fig. S5. MLL1 is uniquely induced in TNF α -programmed DC1. EZH2, Jmjd3, MLL1, G9a, Dot1L, Suv39h1, Dnmt3a, CBP, KDM3b, KDM5d, HDACs 1-6, and HDACs 8-11 were screened for upregulation in TNF α -programmed DC1 by RT-qPCR. N = 18 from three separate, matched experiments. * p < 0.05; ** p < 0.01; *** p < 0.001.

Figure S6

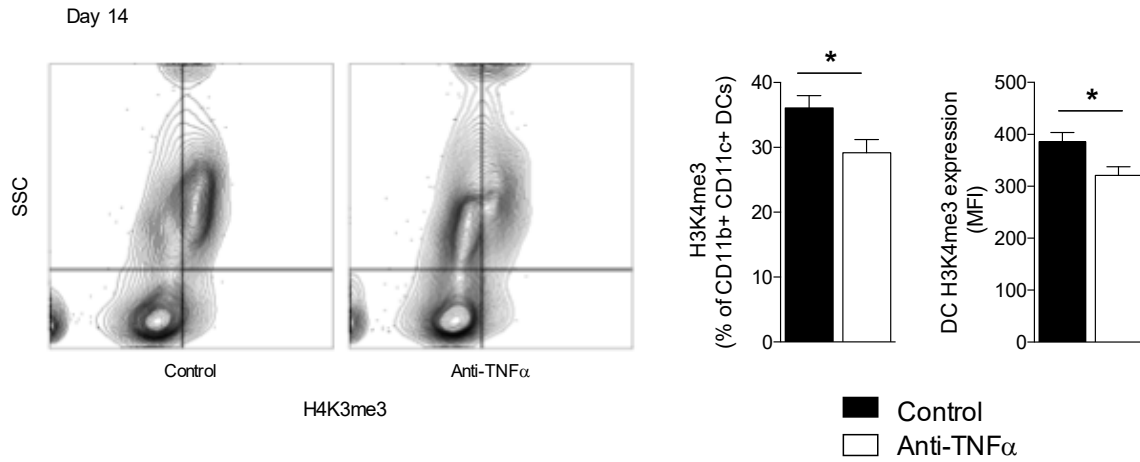


Fig. S6. DC1 from control *C. neo*-infected mice epigenetically resemble

TNF α -programmed DC1. CBA/J mice were infected with 1×10^4 CFU *C. neo* and

sacrificed at days 7, 14, and 28 post-infection. Intranuclear flow cytometry was

performed for H3K4me3 in gated lung DC populations between control and anti-TNF α

mice. N = 8 per group from 2 separate, matched experiments conducted months apart

and from different cages. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Figure S7

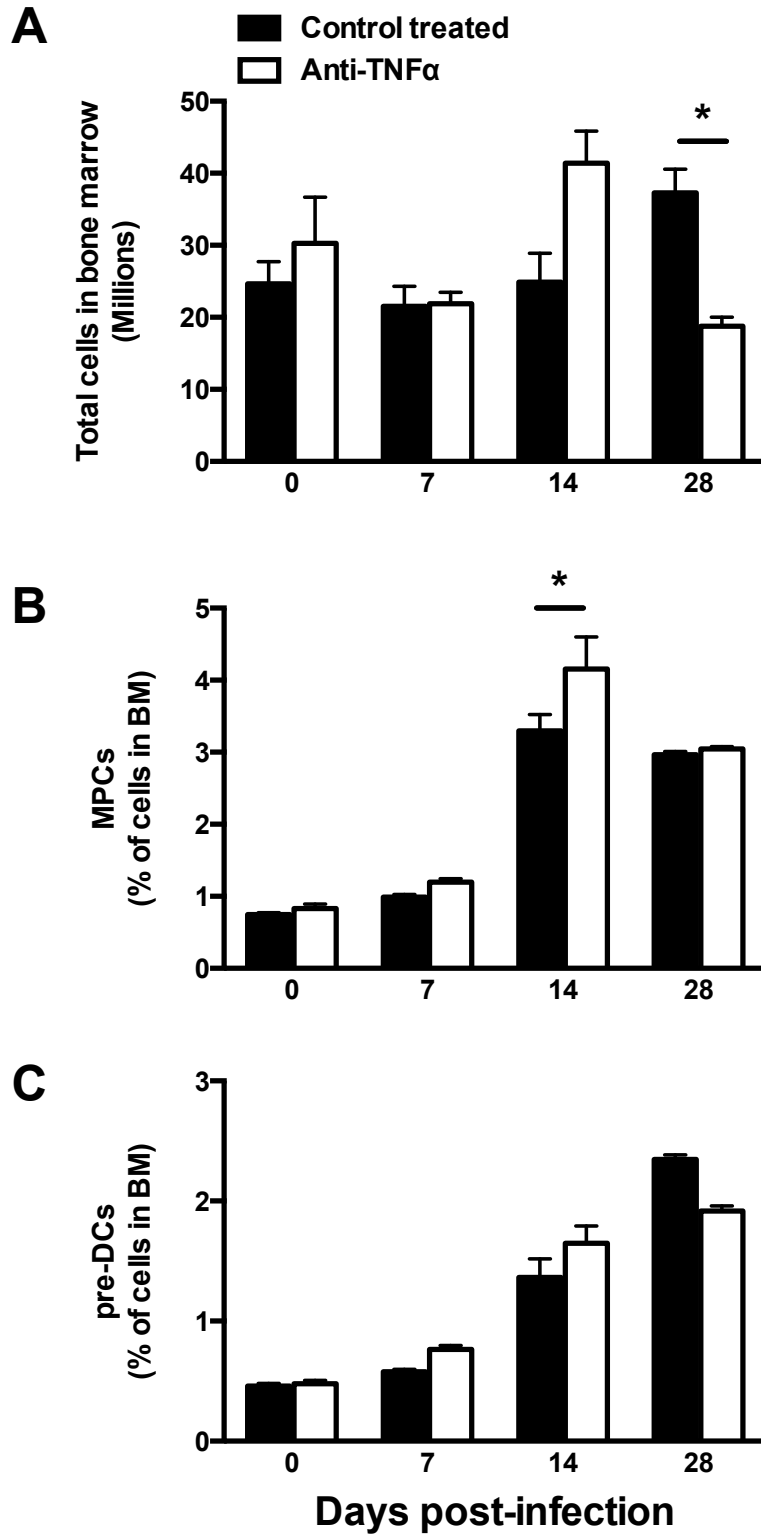


Fig. S7. Assessment of total and myeloid precursor populations in the BM during *C. neo* infection. (A) Mice were infected and/or TNF α depleted as in Figure 1. One femur from each mouse was dissected, the marrow flushed, and total cell numbers enumerated by light microscopy. (B and C) Counted marrow cells were stained for flow cytometric analysis. Myeloid precursor cells (MPCs) were gated as: live/lin-/SCA1-/Flt3+/CD115 high (B) Dendritic cell precursors (pre-DCs) were gated as live/lin-/SCA1-/Flt3+/CD115high/c-kit low (C) Statistical significance was determined by two-way ANOVA with multiple comparisons test or student's t-test where appropriate. * $p < 0.05$; ** $p < 0.01$.