**Immunity, Volume *54***

## Supplemental Information

# The persistence of interleukin-6 is regulated by a blood buffer system derived from dendritic cells

**Ashraf S. Yousif, Larance Ronsard, Pankaj Shah, Tatsushi Omatsu, Maya Sangesland, Thalia Bracamonte Moreno, Evan C. Lam, Vladimir D. Vrbanac, Alejandro B. Balazs, Hans-Christian Reinecker, and Daniel Lingwood**

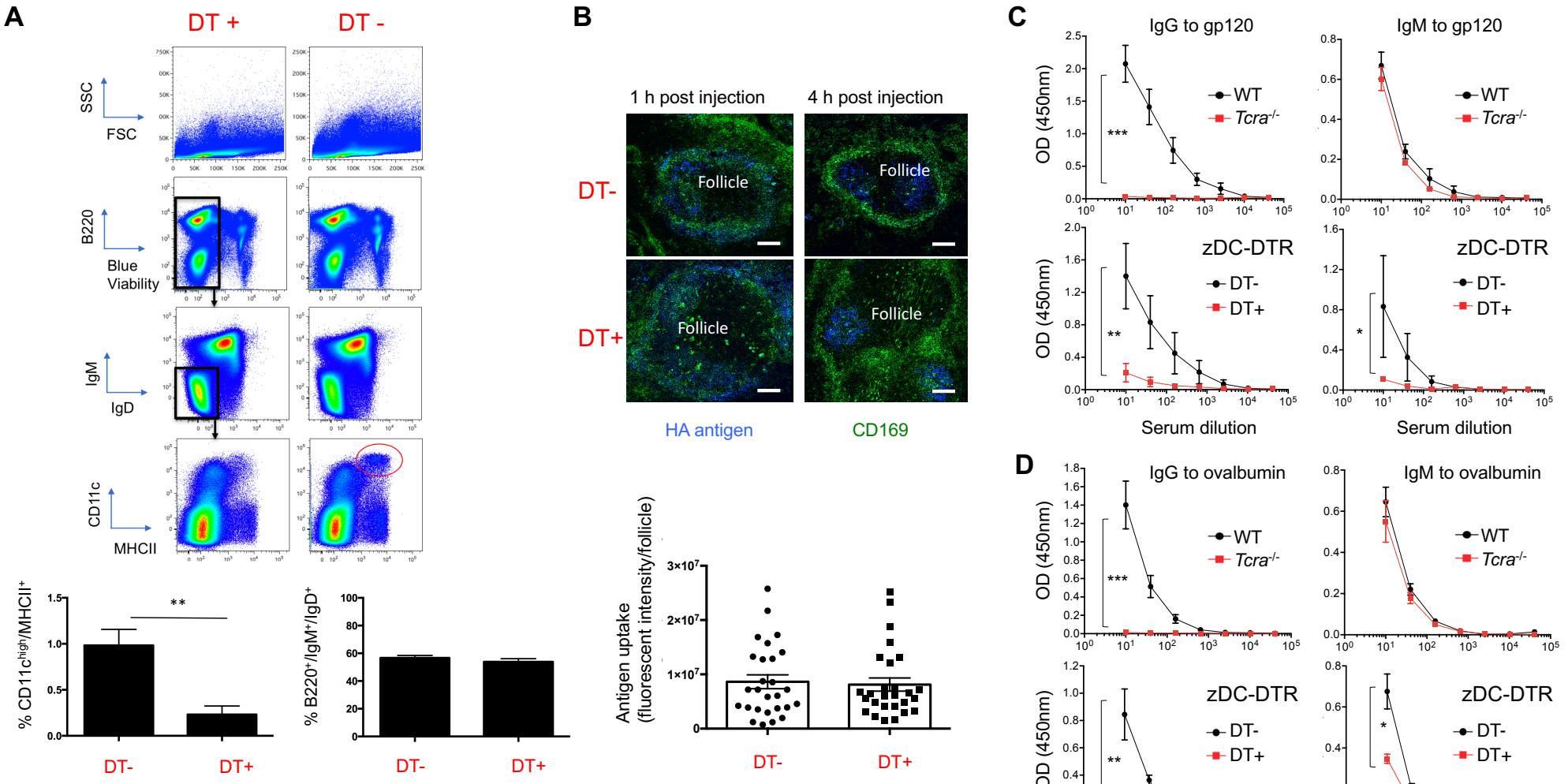


**Figure S1. *Zbtb46*-DTR Chimeras lack cDC but take up antigen from the blood and show cDC-dependent TCR-independent control over the antibody responses to the protein antigens gp120 and ovalbumin, related to Figure 1.** (A) Representative flow plots in untreated and DT-treated chimeras. The cDCs ($B220^-/CD3^-/CD11c^{hi}/MHCII^+$) were reduced upon addition of DT (presented are mean ± SD, n= 3 animals per group; **P<0.002, Student's T test). Naïve B cells ($CD3^-/B220^+/IgM^+/IgD^+$) are also presented in a side-by-side comparison. (B) Capture and uptake of HA-APC following intravenous injection. Spleen follicles were demarcated by CD169 metallophilic macrophages (bar = 50 $\mu$m). The spleen sections were imaged at one and four hours post-injection and the amount of antigen taken into the follicles (intensity per spleen follicle at four hours) within DT treated versus untreated *Zbtb46*-DTR chimeras was quantified by imaging software (10 replicates within each of n=3 DT-treated or n=3 untreated chimeras). (C) Serum IgM versus IgG responses against HIV gp120 in *Tcra*$^{-/-}$ versus WT mice, and *Zbtb46*-DTR chimeras receiving or not receiving DT. (D) Serum IgM versus IgG responses against ovalbumin in *Tcra*$^{-/-}$ versus WT, and *Zbtb46*-DTR chimeras receiving or not receiving DT. (E) Serum IgM versus IgG responses to HA in WT versus *Ifnar1*$^{-/-}$ mice. The dilution curves (presented as mean ± SD, n=5 animals per group), were quantified by endpoint dilution (*P<0.02, **P<0.03, ***P<0.0001, Student's T test).

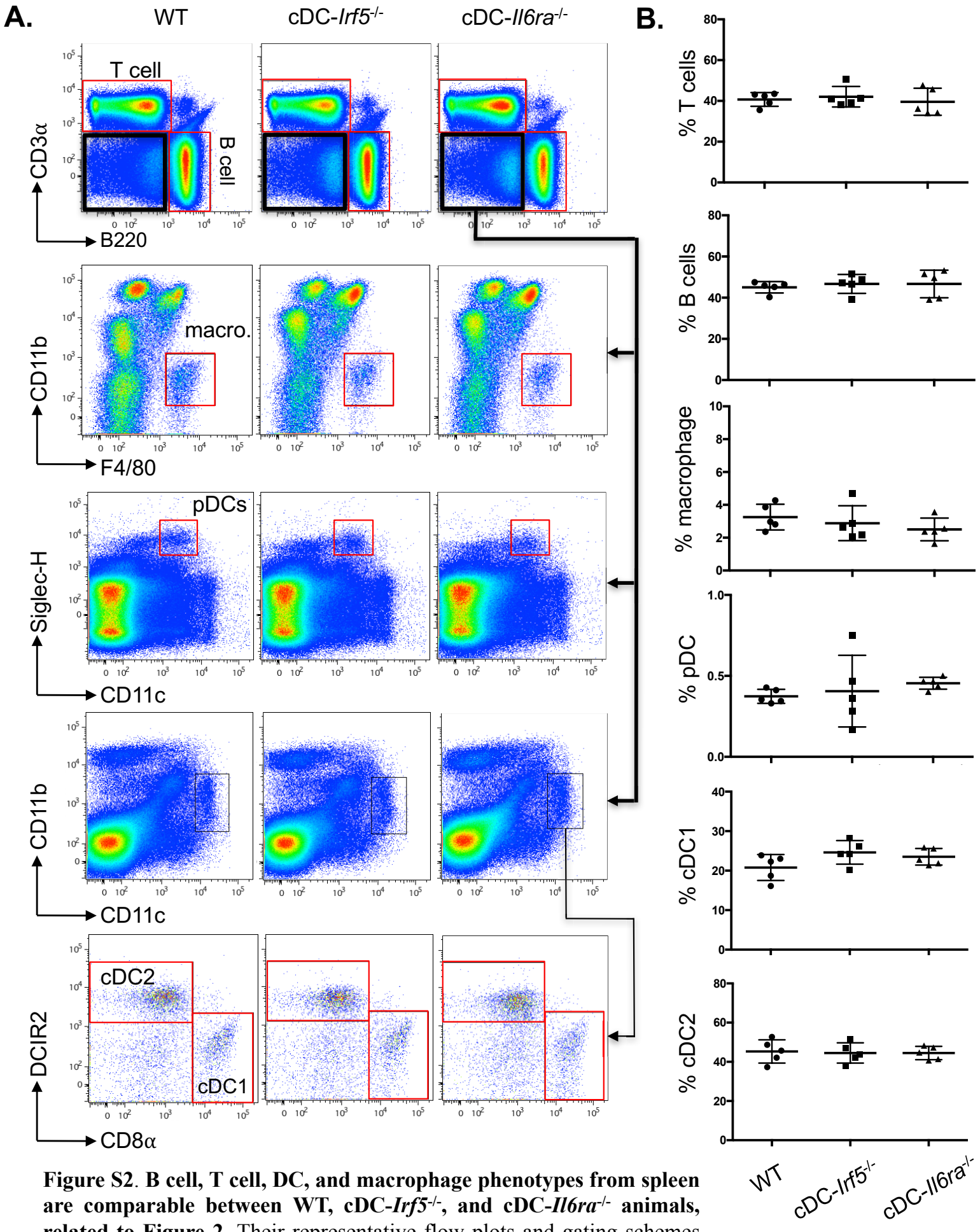


**Figure S2**. **B cell, T cell, DC, and macrophage phenotypes from spleen are comparable between WT, cDC-*Irf5*$^{-/-}$, and cDC-*Il6ra*$^{-/-}$ animals, related to Figure 2.** Their representative flow plots and gating schemes are presented along with a corresponding quantification of proportions of these immune cell types (mean ± SD, n= 5 animals per genotype).

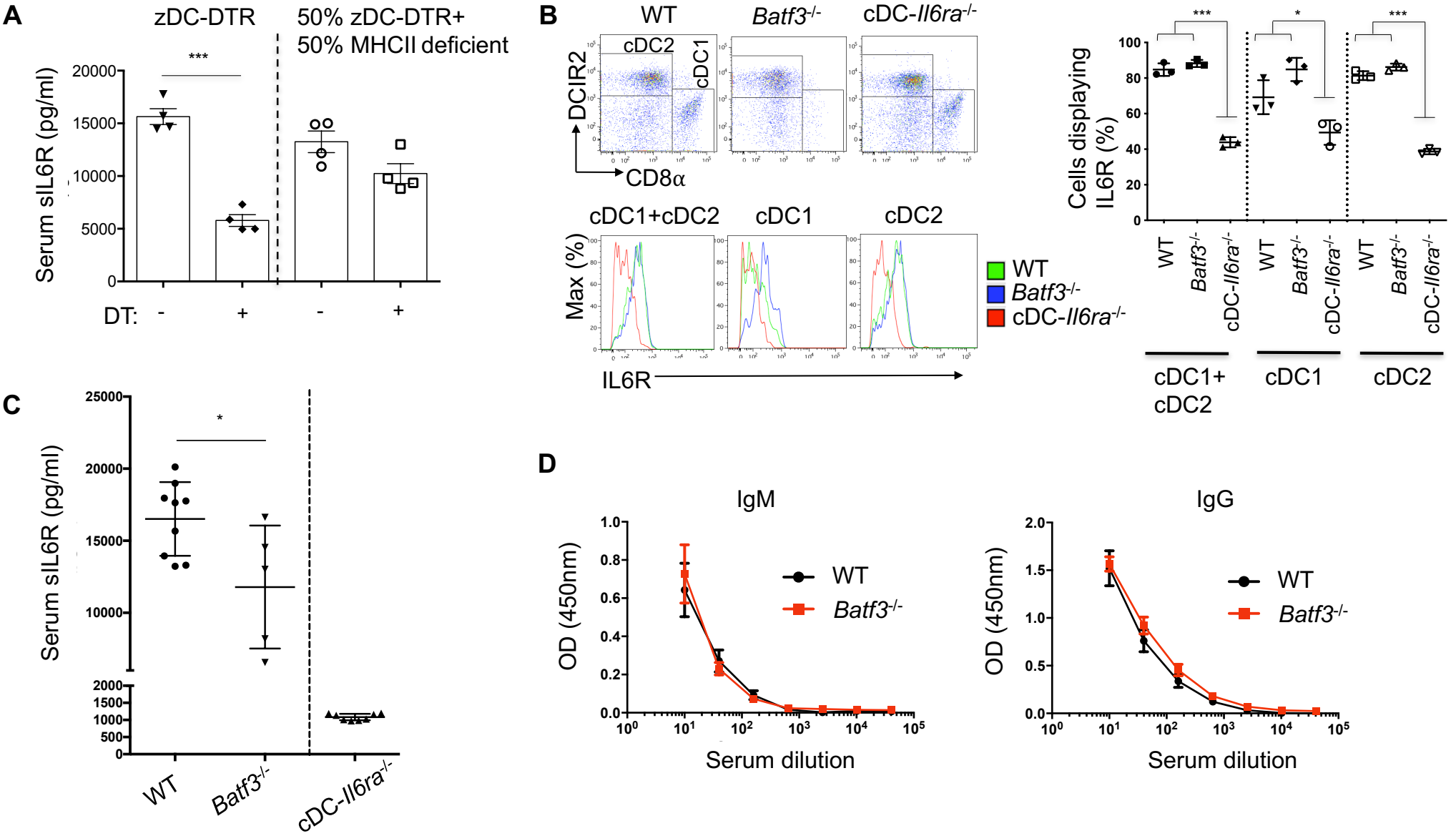


**Figure S3. IL6R measured in bone marrow chimeras and in *Batf3*$^{-/-}$ mice, related to Figure 2**. (A) Concentration of circulating sIL6R in 100% *Zbtb46*-DTR chimeras or 50% *Zbtb46*-DTR +50% MHCII deficient (homozygous *H2*$^{dlAb1\text{-}Ea}$), each ± DT. Presented is the mean ± SD where ***P<0.001, Student's T-test. (B) Surface expression of IL6R in cDC1 and cDC2 within WT, *Batf3*$^{-/-}$ (in which cDC1 is largely absent), and cDC-*Il6ra*$^{-/-}$ (*P<0.05, ***P<0.001, ANOVA with Tukey's Test). Total cDC is made up by 27.7±2.54% cDC1 and 72.0±2.50% cDC2 (see also Figure S2). (C) Serum sIL6R was measured in WT, *Batf3*$^{-/-}$, and cDC-*Il6ra*$^{-/-}$ genotypes (mean ± SD where *P<0.05, Student's T test). (D) The modest reduction in serum sIL6R seen in the *Batf3*$^{-/-}$ genotype does not impact IgM or IgG responses to HA, as measured in WT vs *Batf3*$^{-/-}$ mice.

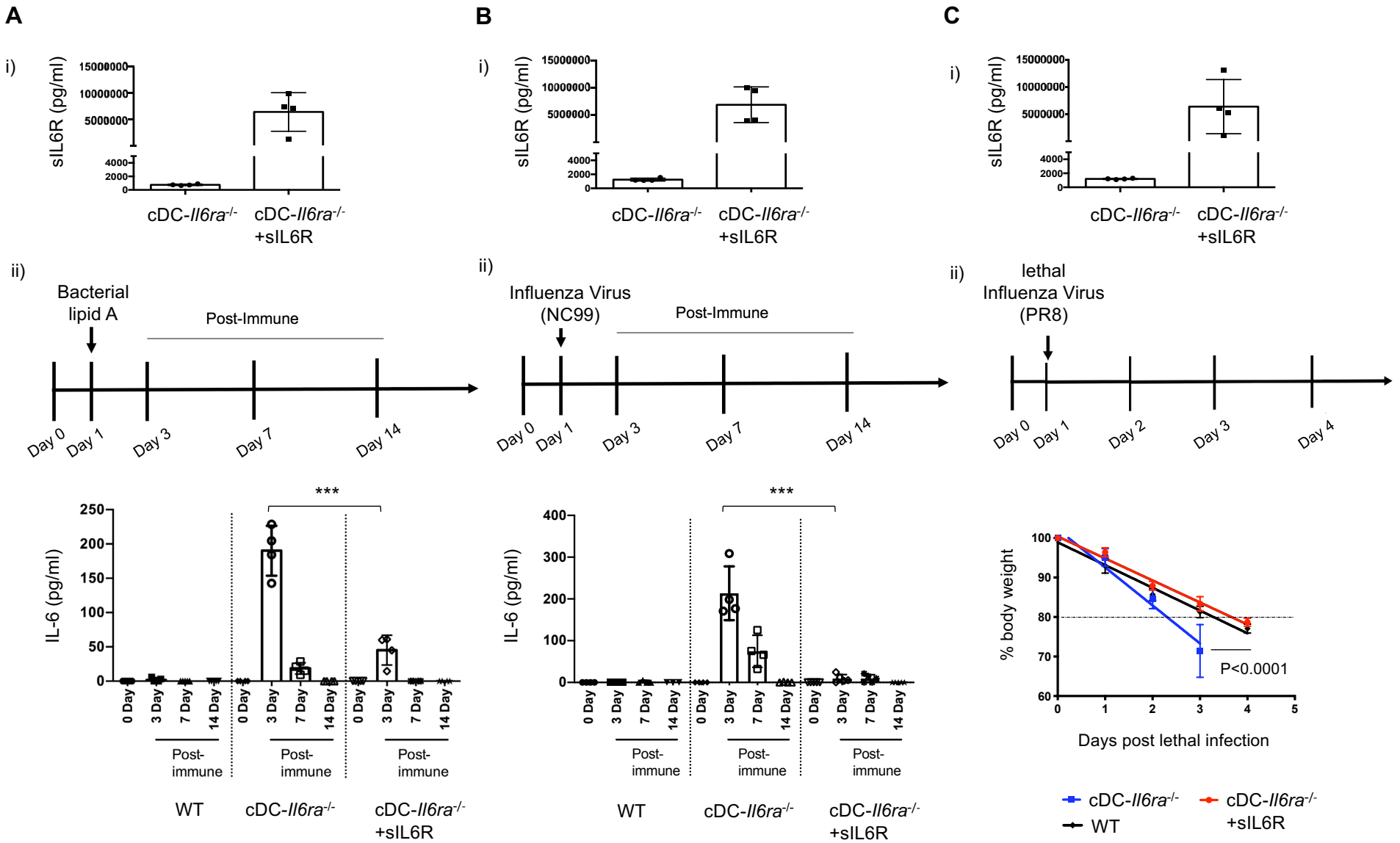


**Figure S4. Addition of sIL6R can rescue dysregulated IL-6 capture in vivo, related to Figure 4.** (Ai, Bi, Ci) sIL6R concentration in cDC-*Il6ra*$^{-/-}$ versus mice of the same genotype that received AAV-sIL6R (three weeks post delivery). (Aii) Animals were then injected with intravenously with bacterial lipid A to promote IL-6 release. The circulating concentration of IL-6 was measured at days 3, 7 and 14 (***P<0.001, ANOVA, with Tukey's Test). (Bii) Animals were infected with NC99 influenza virus to promote IL-6 release. The circulating concentration of IL-6 was measured at days 3, 7 and 14 (***P<0.001, ANOVA, with Tukey's Test). NC99 infects and propagates within mice but does not cause disease. (Cii) Animals were challenged with a lethal dose of PR8 influenza virus and morbidly was indexed by measuring weight loss over the next four days. The rate of decline (slope) was compared using F Tests. If body weights were 80% or less (horizontal bar) the mice were euthanized.