

Supplemental Figure 1. TLR4 deficiency does not impair autoimmunity in D1L3^{KO} mice.

In panels A-E, 1-year-old mice deficient for *Dnase1/3* (D1L3^{KO}, brown) *Tlr4* (TLR4^{KO}, grey) or both (D1L3/TLR4^{KO}, blue) were examined along with wild-type controls (WT, white).

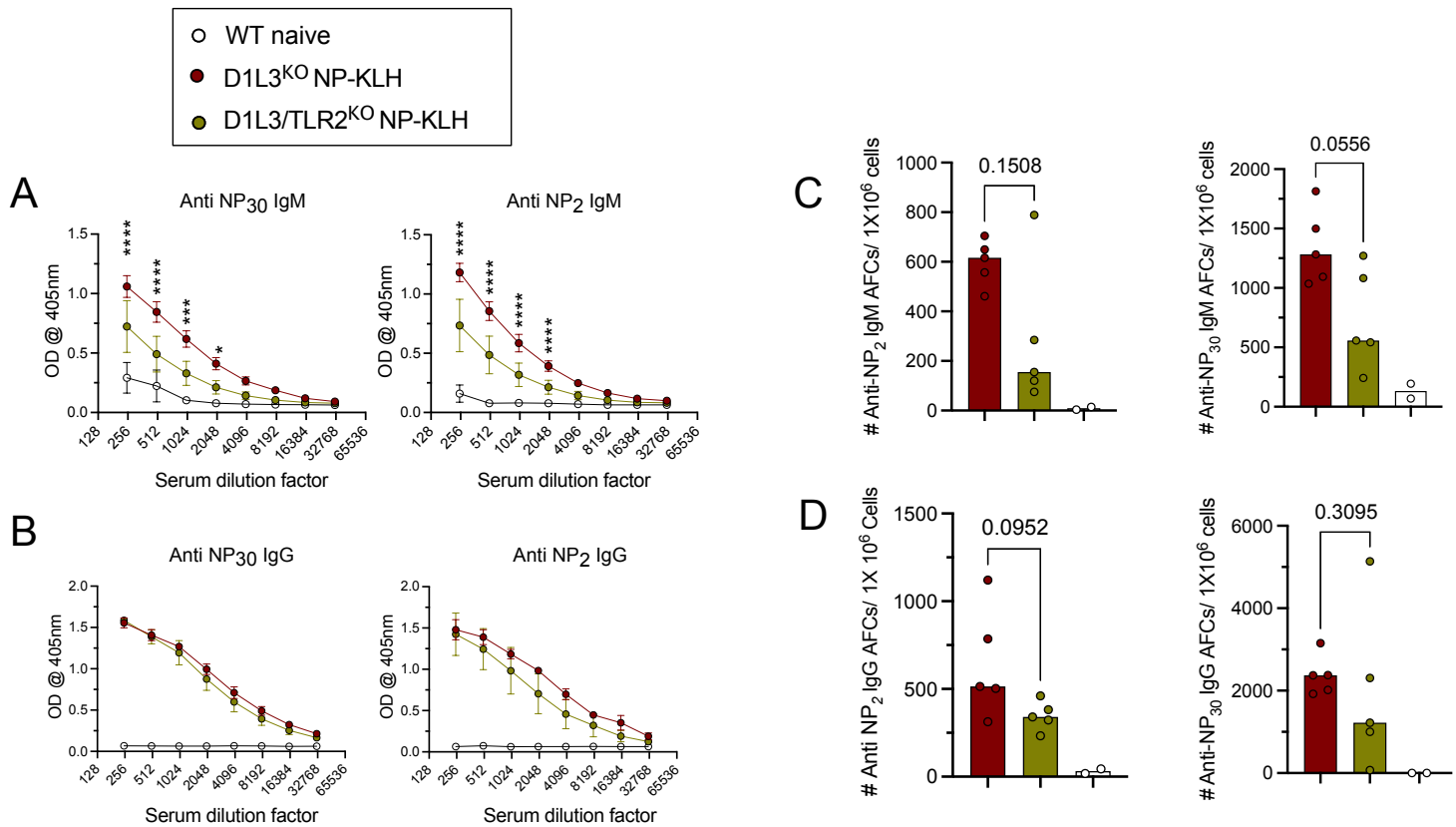
(A) Frequency of class-switched IgG2a2b⁺ IgM⁻ IgD⁻ B cells in the spleen. (B, C) Serum anti-dsDNA IgG titers (B) and anti-Nuc titers (C), at one year of age, as measured by ELISA. (D) Representative images of ANA staining. (E) The distribution of ANA reactivity patterns.

In panels F-M, 1-year-old mice deficient for *Dnase1/3* (D1L3^{KO}, brown) *Tlr2* (TLR2^{KO}, grey) or both (D1L3/TLR2^{KO}, green) were examined along with wild-type controls (WT, white).

(F) Fraction of splenic CD19⁺ TCRβ⁻ CD138⁺ cells with CXCR3⁺ MHC-II^{hi} cells (ExFO B cells). (G) Fractions of CD38⁻ GL-7⁺ GC B cells among total CD19⁺ B220⁺ splenic B cells. (H) Representative flow cytometry plots showing the gating strategy and frequency of CD11b⁺ Ly6C⁻ cells and CD11b⁺ Ly6C⁺ cells among PBMCs.

(I) Fractions of CD19⁺ B220⁺ CD44^{hi} Sca1^{hi} activated B cells. (J, K) Fractions of CD4⁺ CD62L⁻ CD44^{hi} Sca1^{hi} effector T cells and CD4⁺ CD44^{lo} CD62L^{hi} naïve T cells among splenic CD4⁺ T cells. (L) Fraction of CD62L⁻ PSGL-1^{lo} ExFO Th cells among splenic B220⁻ TCRβ⁺ CD4⁺ T cells. (M) Fraction of CXCR5^{int} PD-1^{int} Tfh population among CD4⁺ splenic T cells from 12-mo-old mice.

Symbols represent individual mice and bars indicate median. Statistical significance: ns, not significant; * $p \leq 0.05$; ** $p \leq 0.01$.



Supplemental Figure 2. T cell-dependent foreign antigen responses are unaffected by TLR2 deficiency in D1L3^{KO} mice.

Mice deficient for *Dnase1/3* (D1L3^{KO}, brown) alone or double-deficient for *Dnase1/3* and *Tlr2* (D1L3/TLR2^{KO}, green) were immunized with NP-KLH in alum and boosted on day 7, while control WT mice (open symbols) were left unimmunized. On day 14, mice were sacrificed, serum and splenocytes were collected for ELISA and ELISpot analysis.

(A-B) Titers of anti-NP₃₀ IgM and anti-NP₂ IgM **(A)**, and anti-NP₃₀ IgG and anti-NP₂ IgG titers **(B)** at varying serum dilutions as determined by ELISA. Statistical significance is shown between *Dnase1/3*^{-/-} and *Dnase1/3*^{-/-}*Tlr2*^{-/-}, calculated using 2-way ANOVA followed by Sidak's multiple comparison test **(C and D)** Frequency of anti-NP₂ and anti-NP₃₀ IgM **(C)**, and anti-NP₂ and anti-NP₃₀ IgG antibody-forming cells (AFCs), per 10⁶ splenocytes as determined by ELISpot. Symbols represent individual mice.