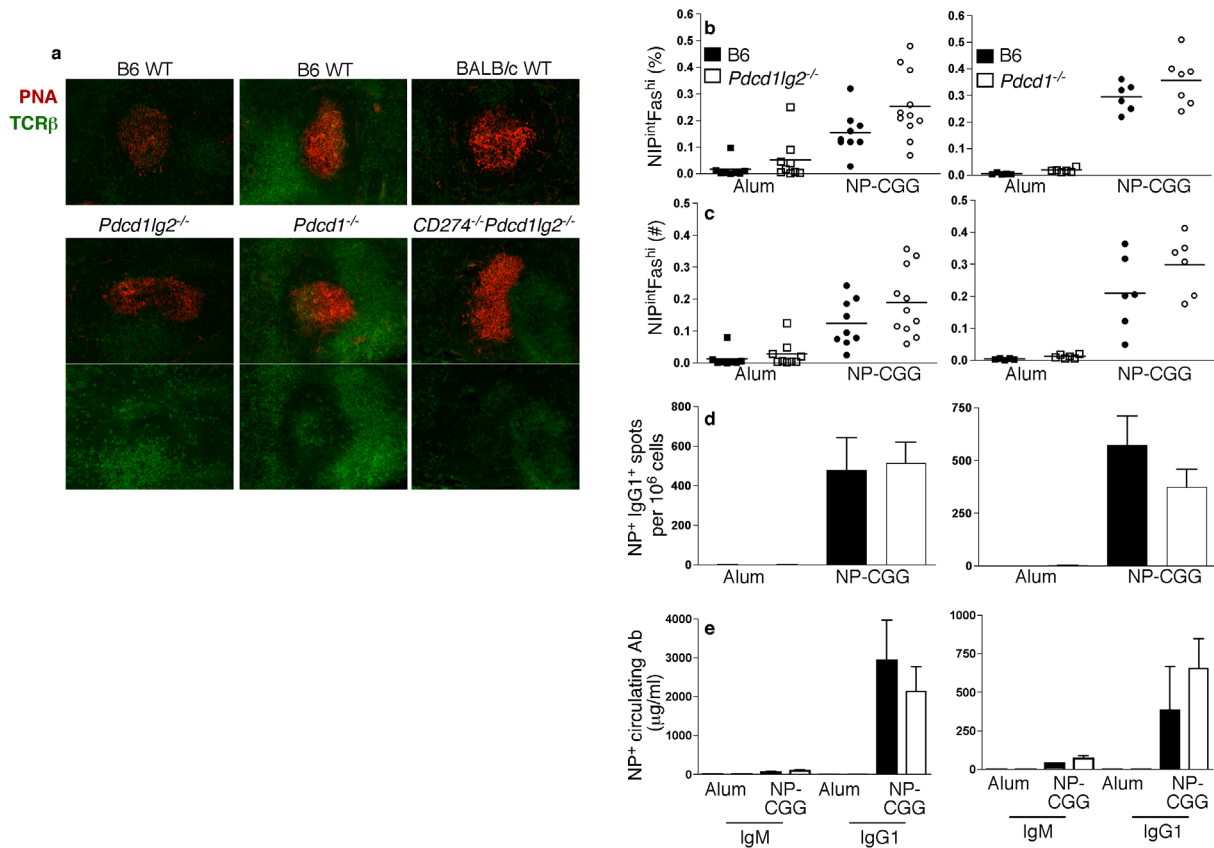


Supplementary Information

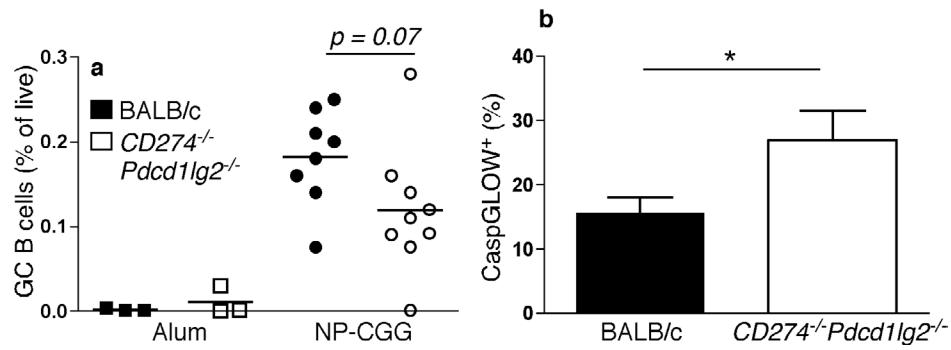
PD-1 regulates germinal center B cell survival and the formation and affinity of long-lived plasma cells

Kim L. Good-Jacobson¹, Courtney G. Szumilas¹, Lieping Chen², Arlene H. Sharpe³ Mary M. Tomayko⁴ & Mark J. Shlomchik¹



Supplementary Figure 1: Induction of the GC response and differentiation of T_{FH} in the absence of PD-1 and its ligands is comparable to WT controls.

Pdc1lg2^{-/-}, *Pdc1^{-/-}*, *CD274^{-/-}Pdc1lg2^{-/-}* or WT controls were immunized with NP-CGG in alum (circles; $n \geq 9$) or alum only (squares; $n \geq 5$), and the GC response assessed 12 days post-immunization. (a) Sections of frozen spleens were stained with Abs to TCR β (green) and PNA (red). (b,c) Percentages and numbers of splenic B cells (CD19⁺EMA⁻) with a GC phenotype (NIP^{int}Fas^{hi}) in *Pdc1lg2^{-/-}* (b) and *Pdc1^{-/-}* (c) mice (open symbols) compared to B6 WT mice (closed symbols) were assessed by flow cytometry. Data are combined from two (b) or three (c) independent experiments. (d,e) NP⁺IgG1⁺ Ab-forming cells (d) and circulating IgM and IgG1 Ab (e) in *Pdc1lg2^{-/-}* and *Pdc1^{-/-}* mice (open bars) compared to B6 controls (black bars). Data are combined from two independent experiments.



Supplementary Figure 2: Increased cell death in *CD274*^{-/-}*Pdc11g2*^{-/-} mice.

Splenic CD19⁺NIP^{int}Fas^{hi} cells undergoing cell death were revealed by detection of activated caspases using CaspGLOW immediately after cell harvest at d15. Frequencies of splenic GC B cells (**a**) that are CaspGLOW⁺ (**b**; $n = 8-9$). Data are combined from two independent experiments.


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358 . . . . . ACA . . . . . CGT TTC . . . . .
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340 350
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Supplemental Figure 3: Vh sequencing of memory B cells.

Memory B cells were sorted from either B6 (a) or *Pdcd1*^{-/-} (b) mice at least 12 wks post-immunization with NP-CGG using the following markers after doublet discrimination: PI^{neg}CD19⁺NIP⁺IgG1⁺CD38⁺kappa^{lo}. Cell pellets were digested overnight and Vh186.2/JH2 sequences were amplified by nested PCR, cloned and sequenced as described in the methods section. Shown are sequences aligned by the Clustal W method.