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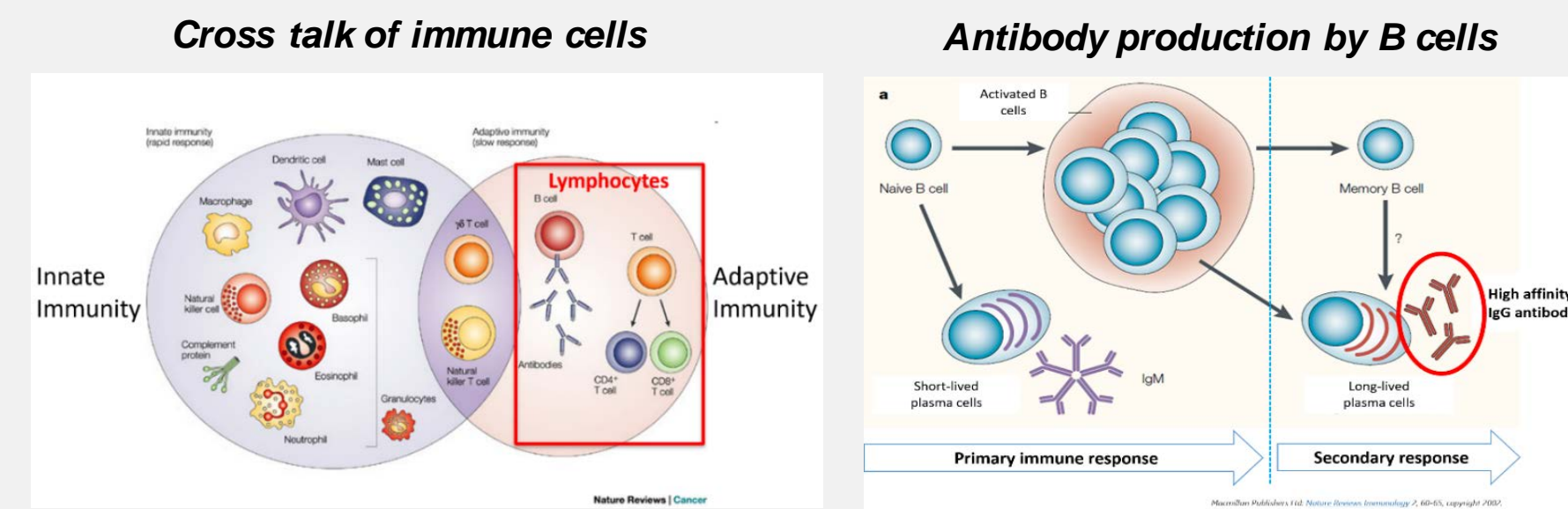
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Abstract

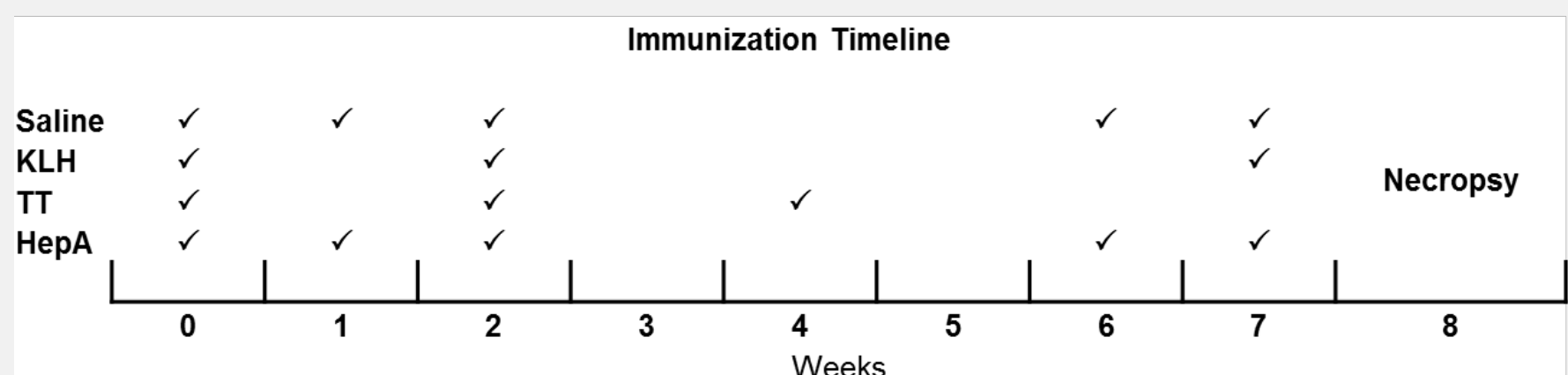
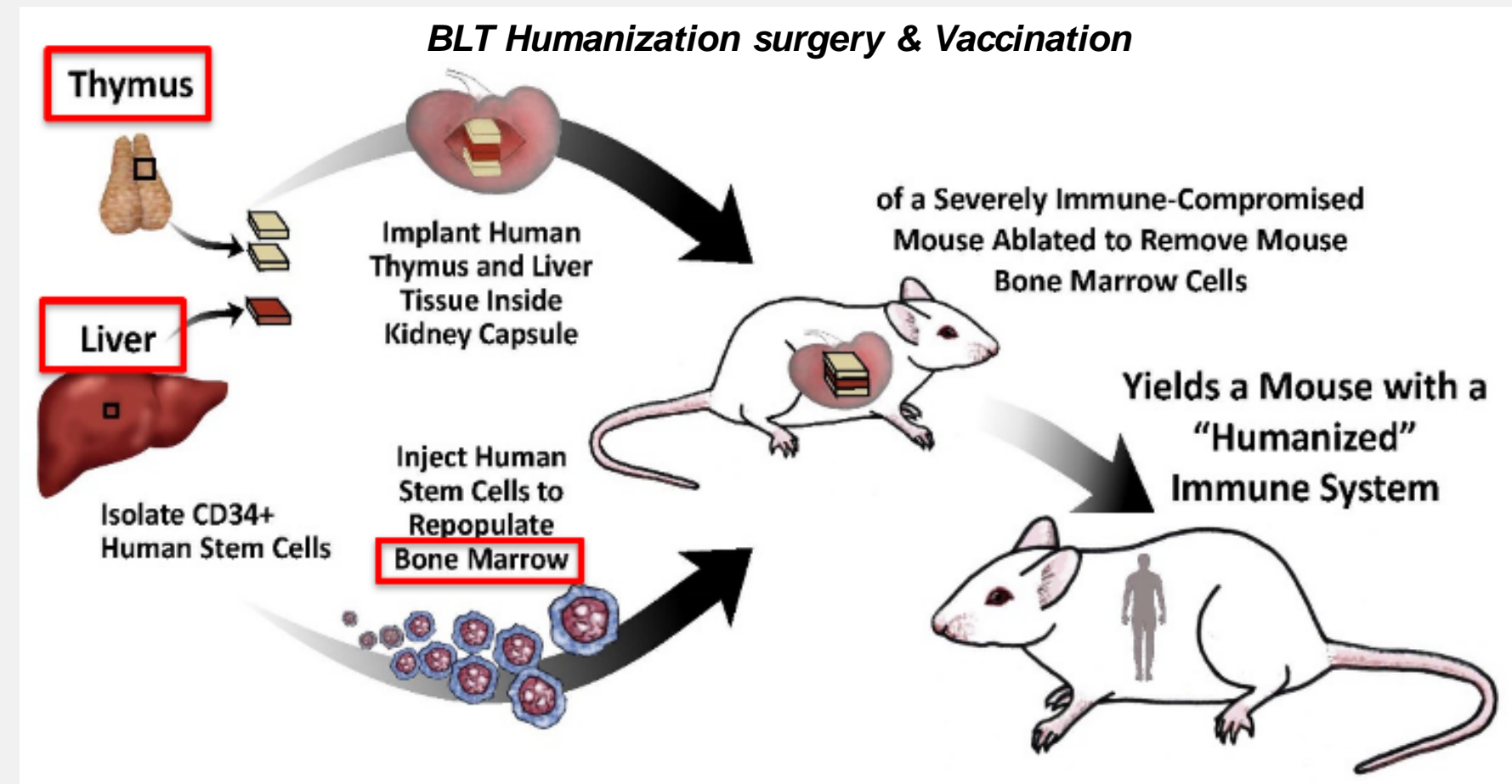
Biologics and drugs can elicit unwanted immune responses affecting patient safety and drug efficacy and animal models are needed to understand adverse human responses. Humanized mice have been shown capable of modeling immune toxicity, but some models show inadequate adaptive immune responses, specifically lacking class-switched, antigen-specific antibody formation. The goal of this study was to evaluate humanized mouse models for T-cell dependent antibody responses. We compared strain-specific antibody responses using bone marrow-liver-thymus (BLT) immune humanized mice made from four unique strains of immune compromised mice (NOG, NOG/hGM-CSF/hIL-3, NOG/hIL-6, and NCG). Following humanization, BLT humanized mice were immunized either keyhole limpet hemocyanin (KLH), Tetanus toxoid (TT), Hepatitis A vaccine or saline to test antigen-specific stimulation. Following immunizations, antibody class-switching and specificity were evaluated using ELISA (enzyme-linked immunosorbent assay) and flow cytometry. Results show that NOG/hGM-CSF/hIL-3 and NOG/hIL-6 strains support improved B-cell production and maturation compared to other strains. Further, in contrast to previously published data, NOG/hGM-CSF/hIL-3 and NOG/hIL-6 strains show development of humoral immunity, supported by the presence of typical B-cell subsets (switched memory B cells and plasma cells) and enhanced isotype-switched, antigen-specific IgG response to immunization. In conclusion, we show an improved preclinical humanized mouse model with more complete humoral immune function. Future studies will use these models to investigate humoral immune response to generic peptide drugs to better understand immunogenicity and adverse events in patients.

Background



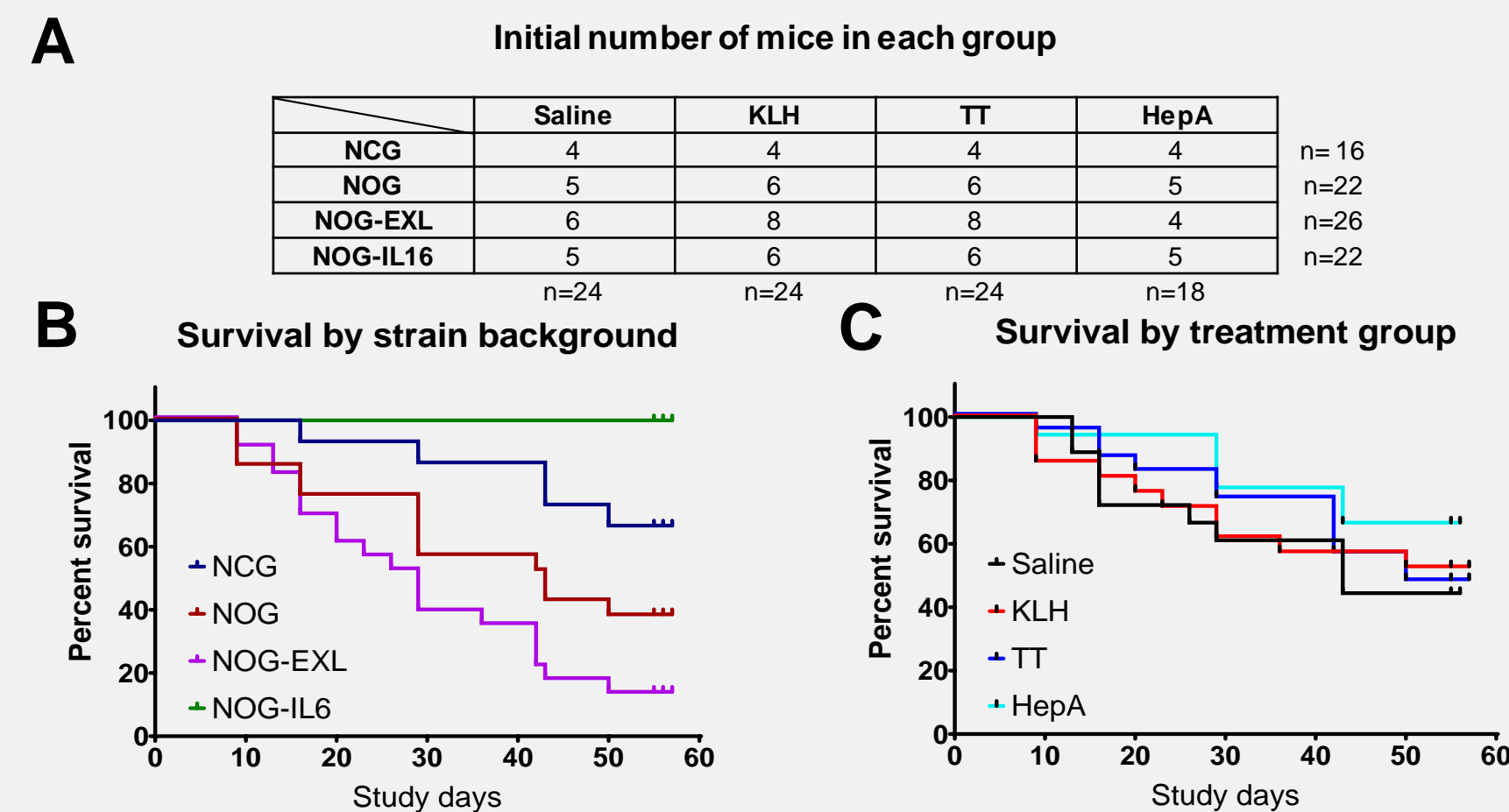
Within the human immune system, different populations of immune cells communicate with each other in order to mount a multi-faceted response to danger signals or foreign antigens. Antibody production by B cells is the hallmark of an immunogenic response. The maturation of the B cell response to produce high-affinity antibodies and memory cells requires additional crosstalk between B cells, antigen-presenting cells (APCs), and T cells.

Study design



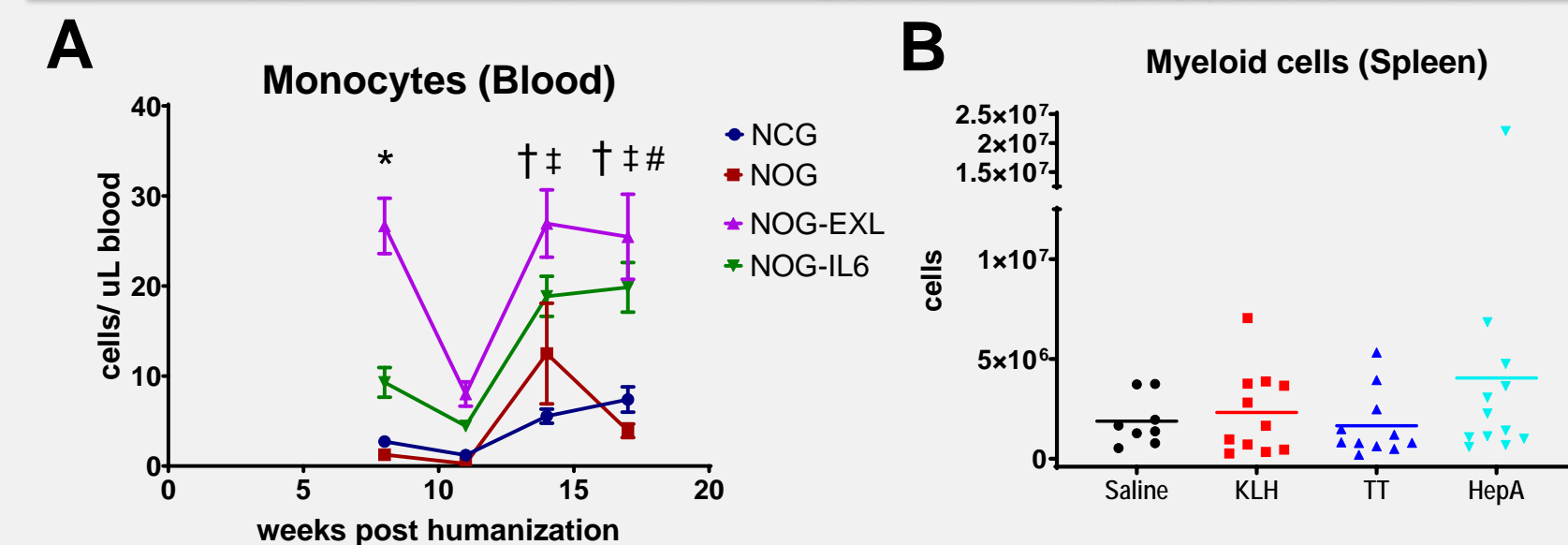
Following humanization, BLT humanized mice were immunized either keyhole limpet hemocyanin (KLH), Tetanus toxoid (TT), Hepatitis A vaccine or saline to test antigen-specific stimulation. Following immunizations, antibody class-switching and the presence of typical B-cell subsets were evaluated using flow cytometry.

Survival after immunization challenge in humanized mice



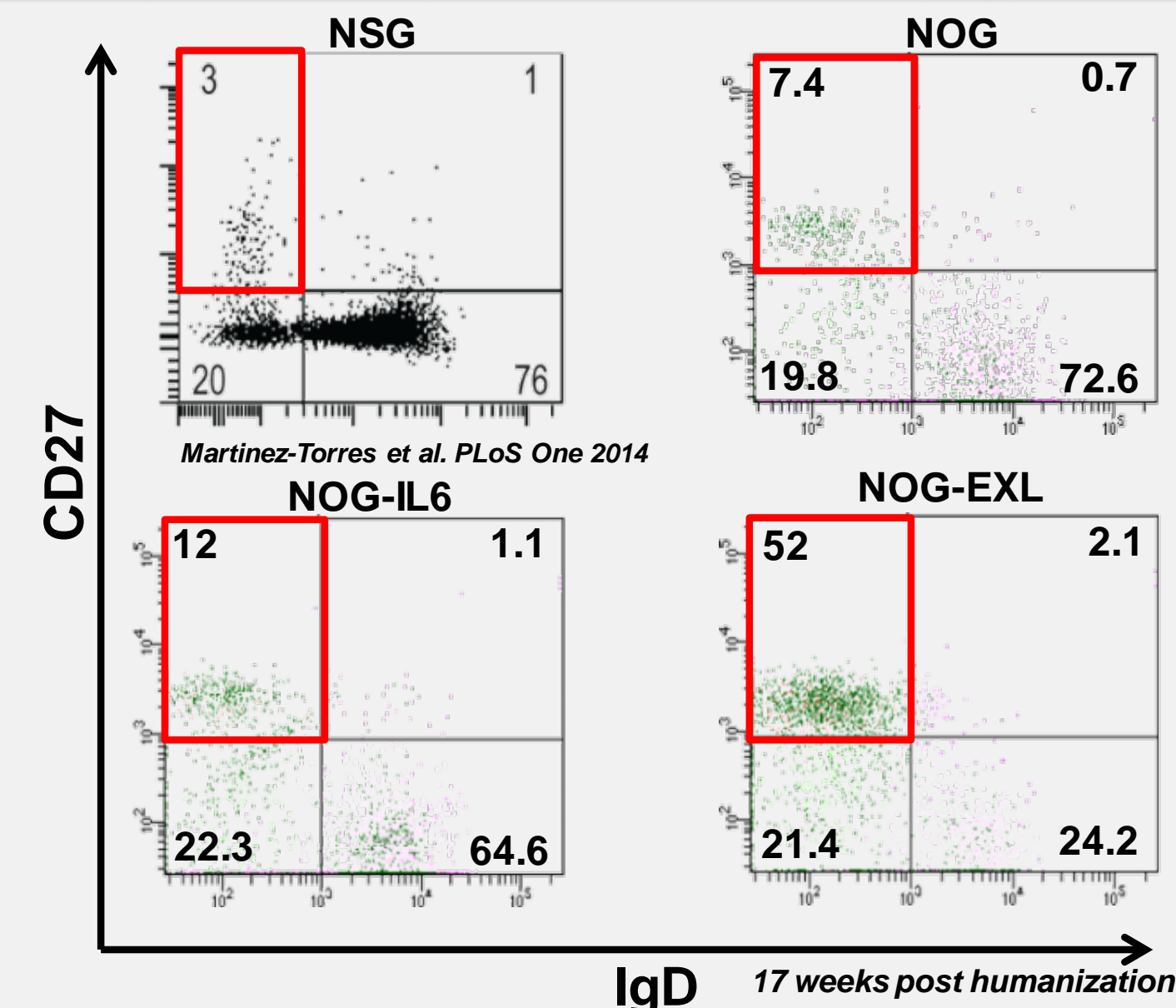
Kaplan-Meier survival curves after immunization challenges. A) Initial number of mice included in each group based on strain background is shown. B) Cumulative survival rate in each strain background shows strain background can significantly influence post-immunization survival. Improved survival rate in NOG-IL6 and NCG strains, compared to NOG and NOG-EXL, was observed. C) A comparison of survival rate across all strains is shown in each treatment group. Effects of treatment on survival were minimal, compared to strain background. Following immunization, NCG (n=16), NOG (n=22), NOG-EXL (n=26), NOG-IL6 (n=22) mice were monitored and euthanized according to approved animal protocols. Mice were purchased from Taconic Biosciences (NOG, NOG-EXL and NOG-hIL6) or Charles River (NCG).

Development of human antigen-presenting cell populations



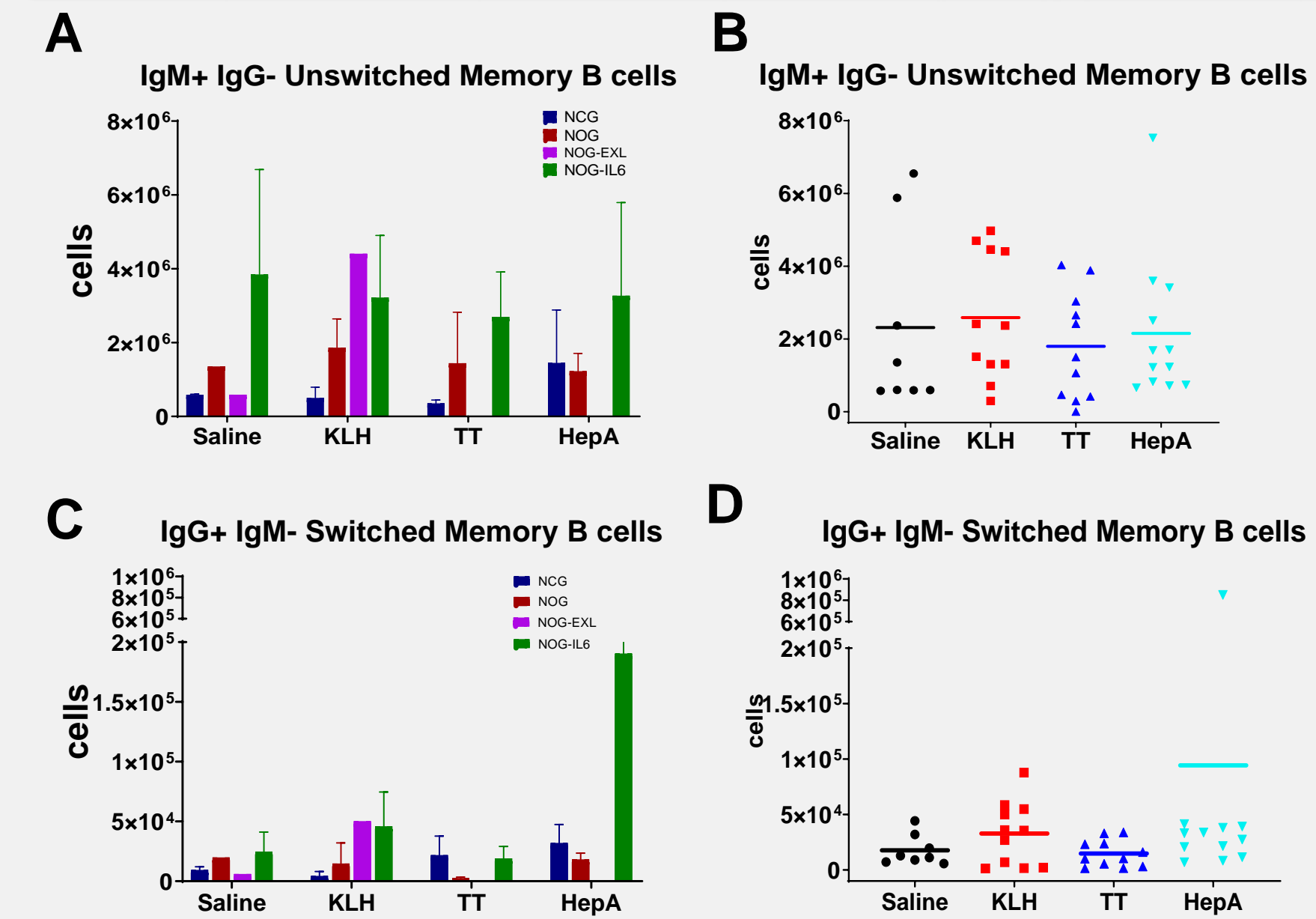
Absolute numbers of human myeloid cell subsets in peripheral blood and spleen of humanized mice. Single cell suspension were stained with antibodies for human CD45, CD19, CD3, CD66b, CD33, and CD14. All cell population in flow cytometry is gated on human CD45+. A) The absolute cell numbers of the CD14+ CD33+ monocyte populations in peripheral blood over the time course of humanization are shown based on humanized mouse strains. Results show that NOG-EXL and NOG-IL6 mice possess increased human myeloid cell numbers following humanization surgery. Statistical analysis was performed via two-way ANOVA, *p<0.05 NOG-EXL vs. NOG & NCG, †p<0.05 NOG-IL6 vs. NCG, #p<0.05 NOG vs. NOG-IL6. B) The CD66b+ or CD14+ myeloid cell population is shown based on treatment group. Myeloid cell population was present in all treatment groups. Statistical analysis was performed via one-way ANOVA with Saline group compared to all other groups.

Identification of mature B cell populations



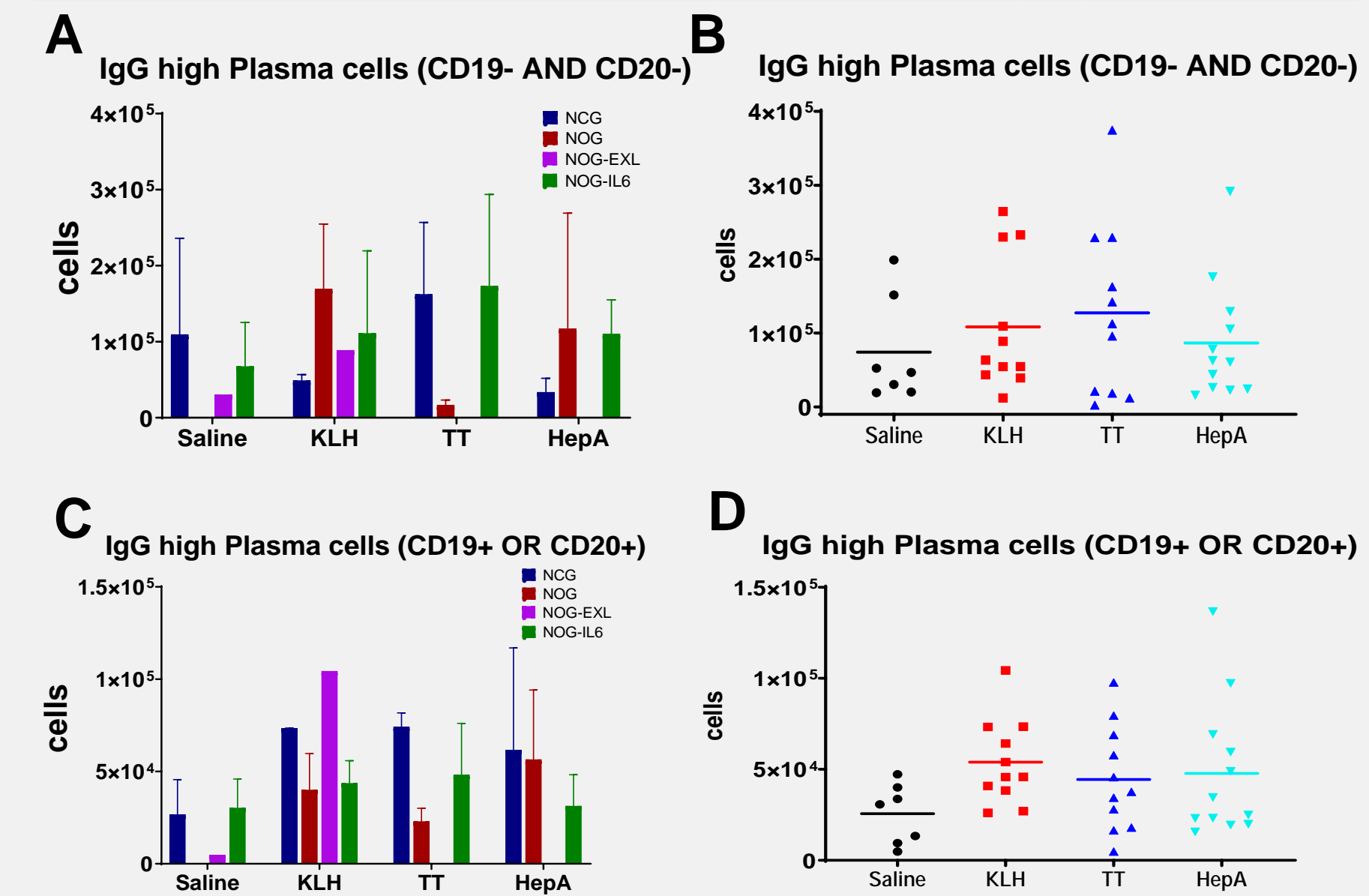
Frequency of mature B cells in peripheral blood of pre-study NSG, NOG, NOG-IL6, and NOG-EXL mice. Single cell suspension of cells of peripheral blood were stained with antibodies for mouse CD45, human CD45, CD19, CD20, IgD and CD27. Flow cytometry dot plots shown are all gated on human CD45+, and CD19+ or CD20+. The frequency of the CD27+IgD- activated mature B cell subset population (as a percentage of CD19+ or CD20+ cells) is shown per mouse strain at 17 weeks post humanization. Results show that NOG-EXL mice support the increased development and activation of mature human B cells following humanization surgery prior to vaccination.

Identification of isotype switched memory B cells in spleen



Frequency of memory B cells in spleen. Single cell suspension of cells of spleen were stained with antibodies for mouse CD45, and human CD45, CD19, CD20, IgG, IgM, CD40 and IgD. All cells are gated on human CD45+. IgM- IgG+ unswitched memory B cells are shown based on A) strain background B) treatment group. IgG+ IgM- switched memory B cells are shown based on C) strain background D) treatment group. Statistical analysis was performed via one-way ANOVA with *p<0.05 Saline group compared to all other groups.

Identification of IgG antibody secreting plasma cells in bone marrow



Frequency of IgG antibody secreting plasmas cells in bone marrow. Single cell suspension of cells of bone marrow were stained with antibodies for mouse CD45, and human CD45, CD19, CD20, IgG, CD27 and CD38. All cells are gated on human CD45+. IgG high plasma cells gated on CD19- and CD20- are shown based on A) strain background B) treatment group. IgG high plasma cells gated on CD19+ or CD20+ are shown based on C) strain background D) treatment group. Statistical analysis was performed via one-way ANOVA with *p<0.05 Saline group compared to all other groups.

Conclusions

- NOG-IL6 and NCG strains show better survival rates throughout the experiment, compared to NOG and NOG-EXL strains.
- NOG-EXL and NOG-IL6 strains support improved myeloid cell and mature B cell production, compared to the NOG and NCG strains.
- The presence of IgG antibody secreting switched memory B cells and plasma cells in immunized mice show development of robust adaptive immunity in humanized mice.

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Disclaimer

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