SUPPORTING INFORMATION/SUPPLEMENTAL

SDC MATERIAL AND METHODS

Serum anti-A antibody measurement by ELISA:

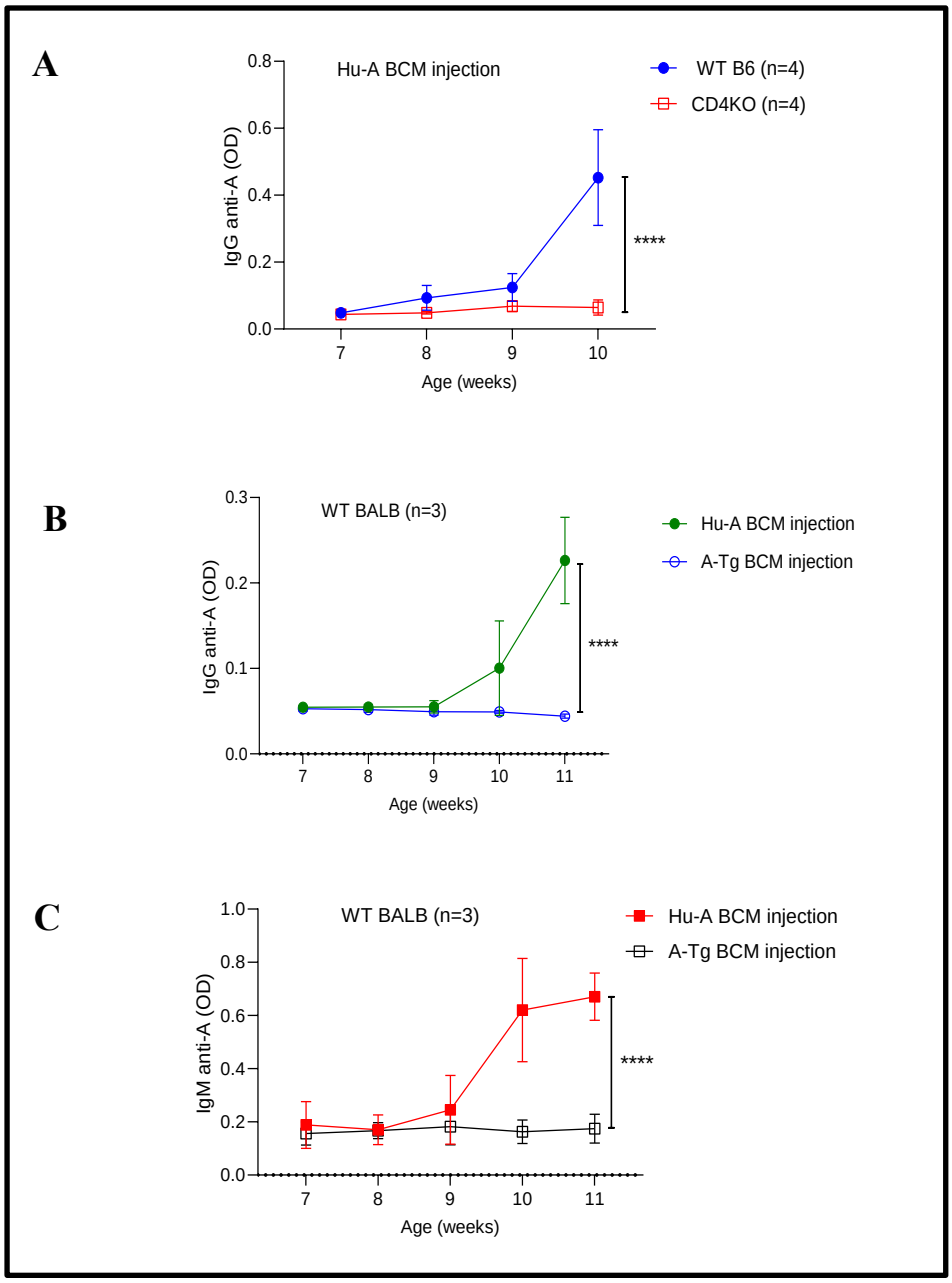
By modifying a method previously published elsewhere [1, 2], transparent flat-bottom Greiner medium-binding ELISA 96-well plates (Sigma-Aldrich, Germany) were coated with 50 µL of standard anti-mouse IgM and IgG (Bethyl Laboratories Inc., USA), or with synthetic A-Ag or B-Ag (A or B-trisaccharides polyacrylamides (A-PAA or B-PAA)) at 5 µg/mL in 0.1M Na_2CO_3 (pH 9.6). The plate was incubated at room temperature for one hour, and then at 4°C overnight. The next day, 5% normal goat serum in PBS was used to block non-specific Ab. Next, 10% diluted mouse serum/plasma, or serially diluted standard mouse reference serum was incubated at room temperature (RT) for 60-90 min. To detect the bound Ab, goat anti-mouse IgM or IgG conjugated with alkaline phosphatase (Bethyl Lab. Inc., USA) was applied for 60 min at RT to detect anti-A/B Abs. Next, p-nitrophenyl phosphate (Sigma-Aldrich, Germany) was used to visualize the phosphatase activity on the secondary Ab at 450 nm.

SDC RESULTS

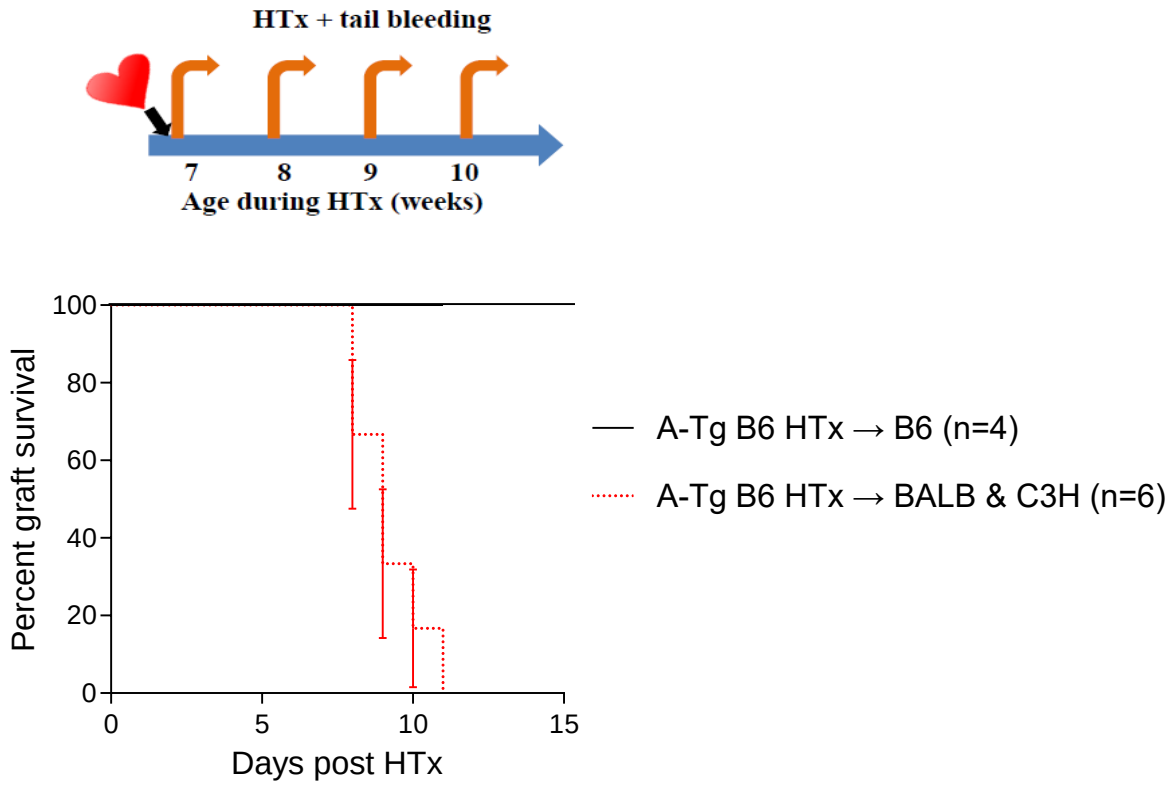
Comparison of untreated or Hu-A BCM-injected B6 mice by sex

WT B6 mice	Untreated (mean titer)	Hu-A BCM injection (mean titer)	p value
Females	4 (n= 34)	1024 (n= 17)	≤ 0.0001 (****)
Males	4 (n= 35)	1024 (n= 22)	≤ 0.0001 (****)
p value	ns	ns	

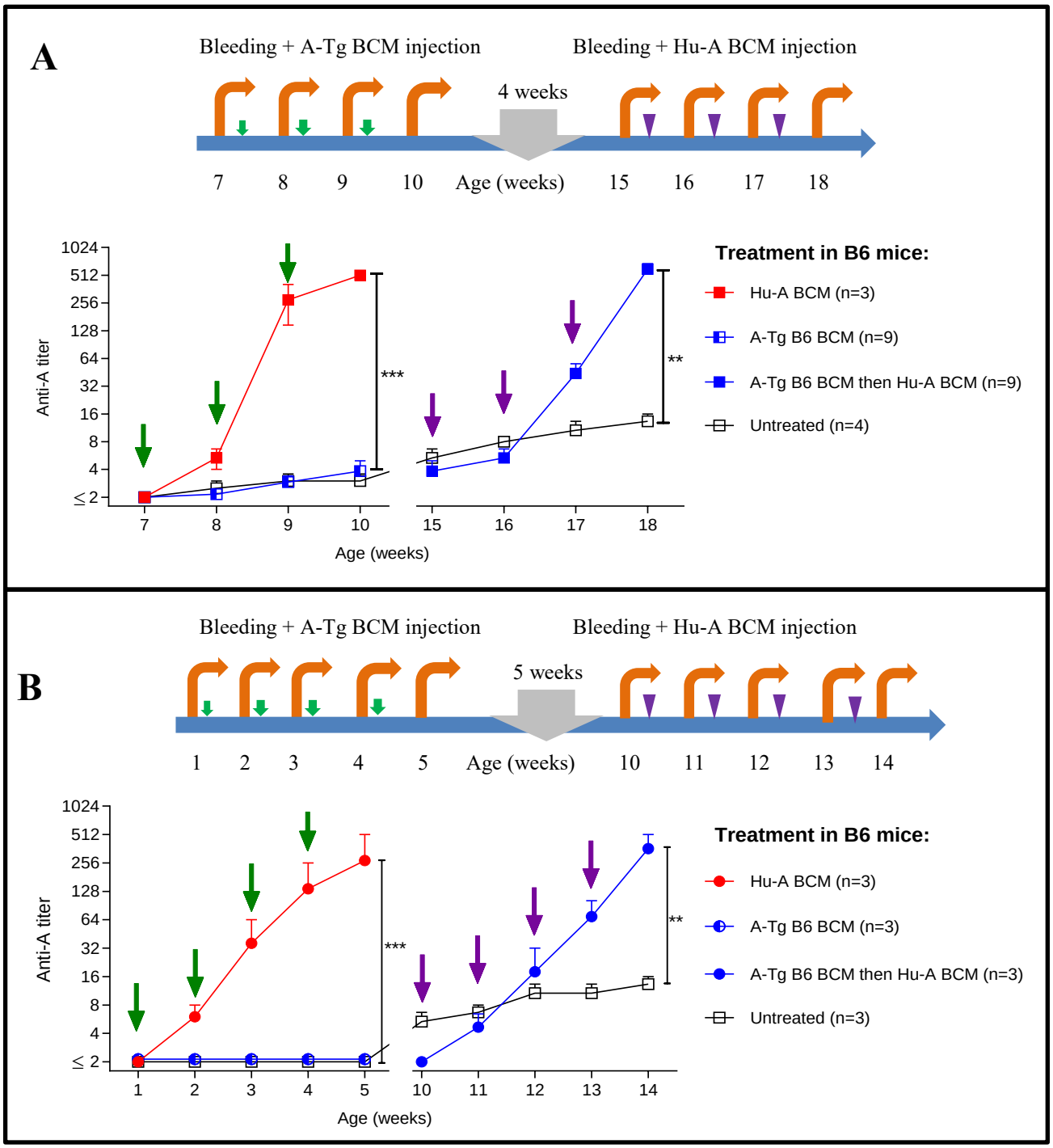
Supplemental Table 1: In 9-10 weeks old B6 mice separated by sex, we compared sera from mice that were either untreated or were injected with Hu-A BCM. *ns=non-significant* ≥0.05*;* **** = $P \leq 0.0001$.



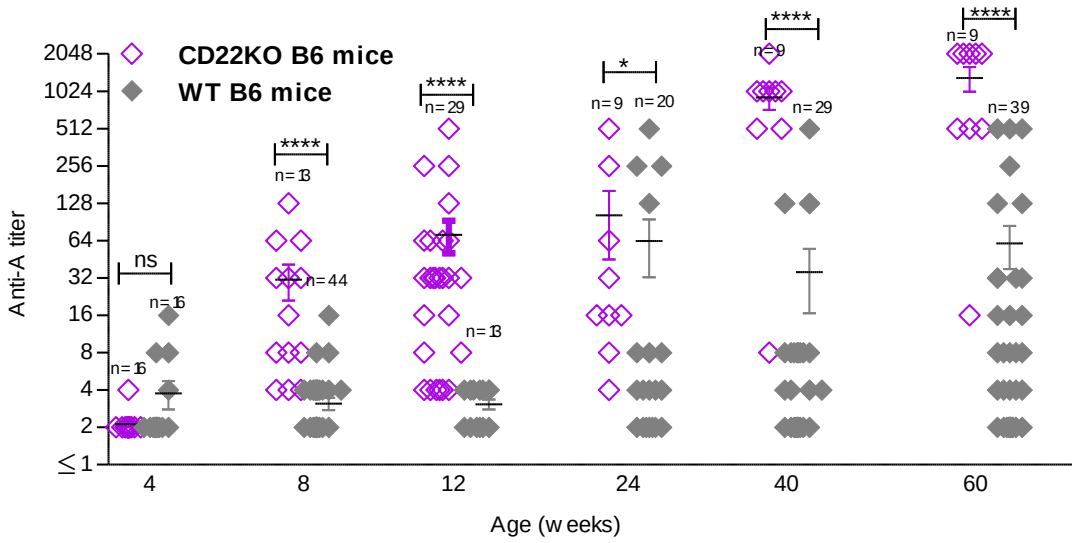
Supplemental Figure 1: WT mice produced both IgG and IgM anti-A in response to stimulation by Hu-A BCM, but not by syngeneic A-transgenic BCM. A) Hu-A BCM induced IgG anti-A in WT B6, but not in CD4KO mice. B) In contrast to injection by syngeneic A-transgenic BALB BCM, Hu-A BCM injection induced IgG B) and IgM C) anti-A in WT BALB females. ELISA assay was used to measure IgM and IgG anti-A antibodies. Data are presented using standard error mean (Mean + SEM) in T-test. **** = $P \leq 0.0001$.



Supplemental Figure 2: Graft survival after A-transgenic heart transplantation (HTx) in syngeneic *vs* allogeneic WT mice. A) A-transgenic B6 heart grafts were transplanted into age- and sex-matched syngeneic (B6) or allogeneic (BALB and C3H) male recipients. Unlike syngeneic grafts, all allografts were rejected within 8-11 days post-transplant. A-Tg: A-transgenic, HTx: heart transplant.



Supplemental Figure 3: Syngeneic A-transgenic BCM did not induce tolerance to subsequent immunization by Hu-A BCM. (A) B6 mice were injected with A-Tg BCM (green arrows, 4 males, 5 females) or Hu-A BCM (1 male, 2 females). Four weeks later, the mice were injected again with Hu-A BCM (purple arrows). (B) One week-old female mice were injected with A-Tg BCM (week 1-4) and four weeks later (weeks 10-13) with Hu-A BCM. Hemagglutination assay was used to measure anti-A titer, using A-transgenic reagent erythrocytes. Data are presented using standard error of mean (Mean + SEM) in T-Test. **: $P \leq 0.01$, ***: $P \leq 0.001$



Supplemental Figure 4: Comparison of anti-A nAb production in CD22KO *vs* WT mice. CD22KO mice (both sexes) produced higher anti-A nAbs than WT mice (both sexes) at all ages. Hemagglutination assay was used to measure anti-A titer, using A-transgenic reagent erythrocytes. Data are presented using standard error mean (Mean + SEM) in T-Test. *ns=non-significant; * = ≥0.05; ** = P ≤ 0.01; *** = P ≤ 0.001; **** = P ≤ 0.0001.*

References:

1. Jeyakanthan M, Meloncelli PJ, Zou L, Lowary TL, Larsen I, Maier S, et al. ABH-Glycan Microarray Characterizes ABO Subtype Antibodies: Fine Specificity of Immune Tolerance After ABO-Incompatible Transplantation. Am J Transplant. 2016;16(5):1548-58.
2. Jeyakanthan M, Tao K, Zou L, Meloncelli PJ, Lowary TL, Suzuki K, et al. Chemical Basis for Qualitative and Quantitative Differences Between ABO Blood Groups and Subgroups: Implications for Organ Transplantation. Am J Transplant. 2015;15(10):2602-15.