

Supplemental material

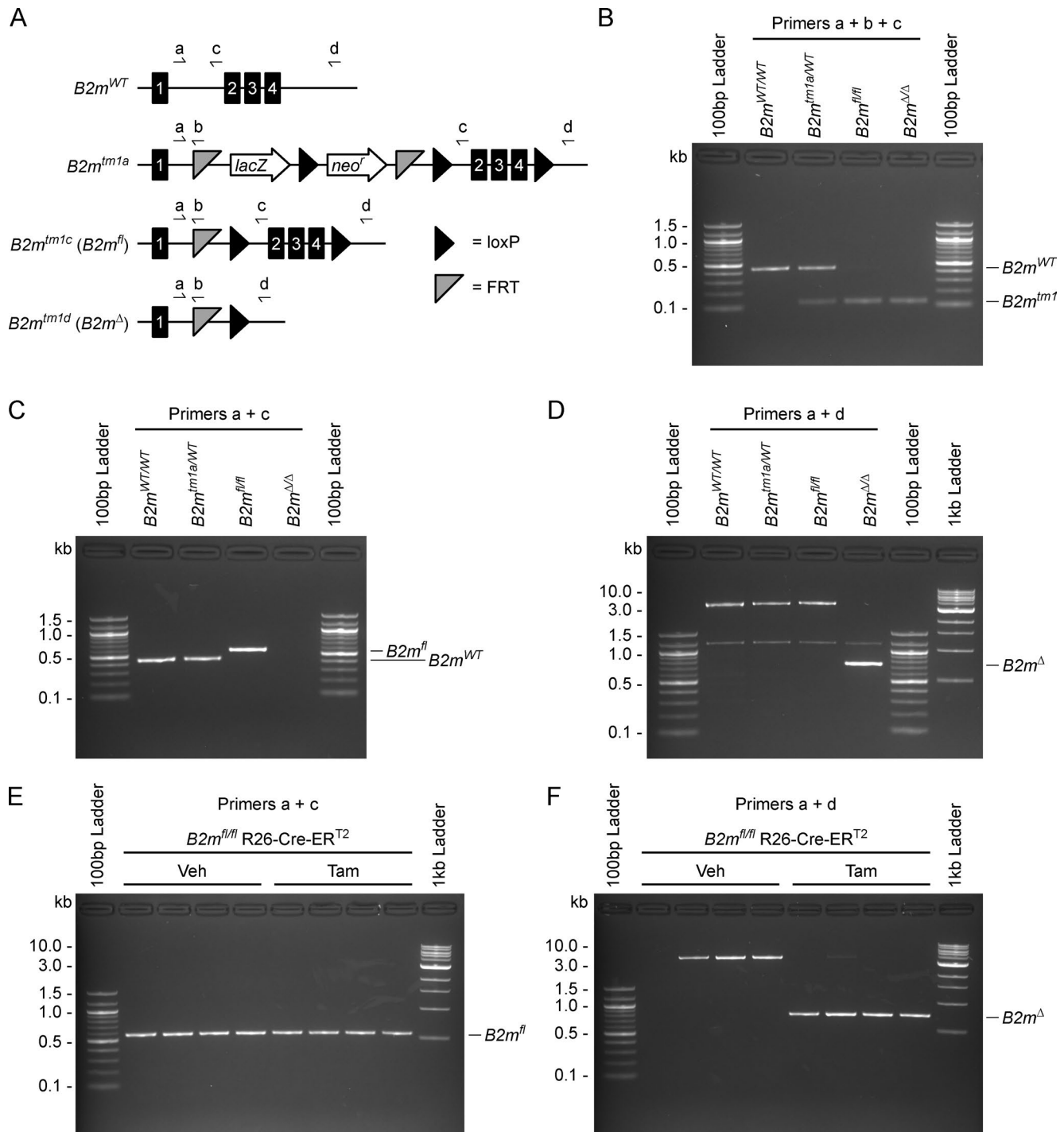
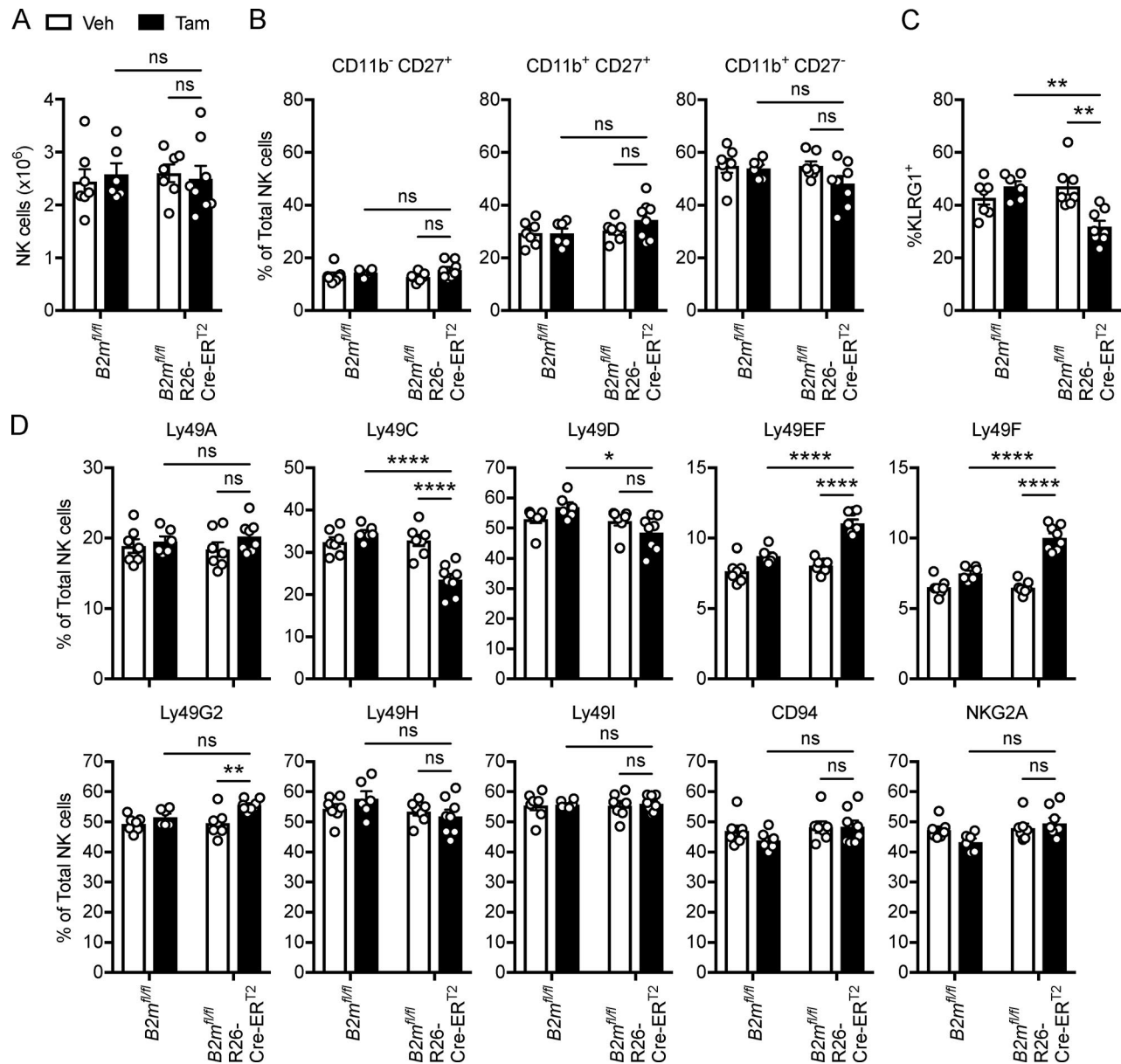
Bern et al., <https://doi.org/10.1084/jem.20181076>

Figure S1. **PCR analysis of genomic DNA from floxed *B2m* mice.** (A–D) Schematic representation of the *B2m* gene as in Fig. 1A with the location of PCR primers a, b, c, and d indicated by arrows. Schematic is not drawn to scale. PCR was performed on genomic DNA isolated from the tails of *B2m*^{WT/WT}, *B2m*^{tm1a/WT}, *B2m*^{fl/fl}, and *B2m*^{Δ/Δ} mice with primers a, b, and c (B); primers a and c (C); and primers a and d (D). (E and F) *B2m*^{fl/fl} R26-Cre-ER^{T2} mice were administered tamoxifen or vehicle control on days 0–4 and tail DNA was isolated on day 14 for PCR with primers a and c (E) or primers a and d (F). The small PCR product in B is labeled *B2m*^{tm1} because it is observed with PCR on *B2m*^{tm1a}, *B2m*^{tm1c} (*B2m*^{fl}), and *B2m*^{tm1d} (*B2m*^Δ) alleles. The large PCR products in D and E were inconsistently observed. Each lane represents an individual mouse. Data are representative of two independent experiments with one mouse per group (B–D) or three to four mice per group (E and F).



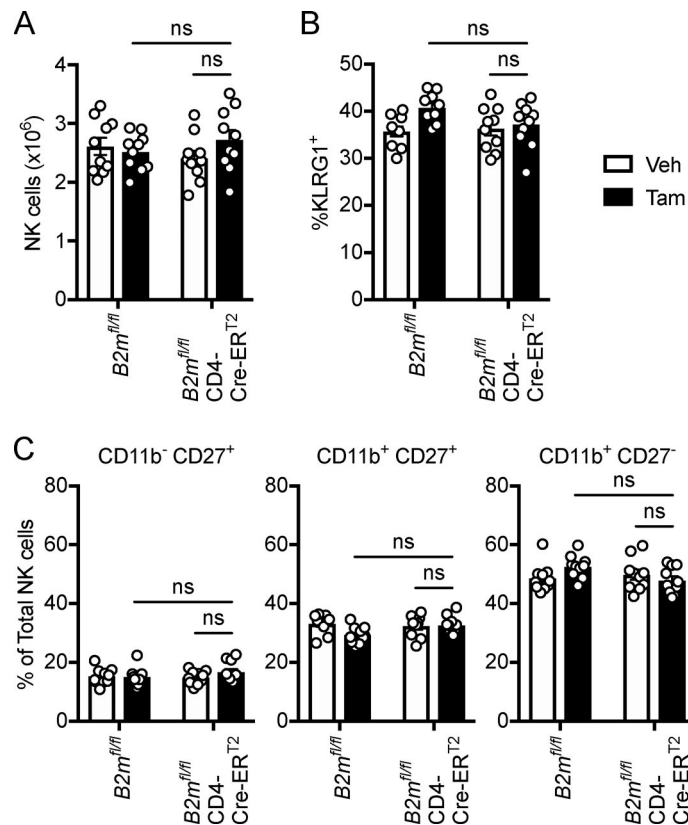


Figure S3. **No changes in NK cell development or maturation are observed after down-regulation of MHC-I on CD4⁺ T cells.** $B2m^{fl/fl}$ and $B2m^{fl/fl} CD4-Cre-ERT2$ mice were treated with tamoxifen or vehicle control on days 0–4, and splenocytes were harvested on day 14 for flow cytometry. **(A)** Total number of splenic NK cells ($CD3^- CD19^- NK1.1^+ NKp46^+$) from the indicated mice treated with vehicle (open bars) or tamoxifen (closed bars; $n = 10$ mice per group). **(B)** The percentage of total NK cells that express KLRG1 ($n = 10$ mice per group). **(C)** The percentage of total NK cells in different maturation stages separated by expression of CD11b and CD27 ($n = 10$ mice per group). Data are combined from three independent experiments. Statistical significance was calculated by two-way ANOVA with Bonferroni's multiple comparisons test. Each symbol represents an individual mouse. Error bars indicate mean \pm SEM; ns, not significant. Tam, tamoxifen; Veh, vehicle.

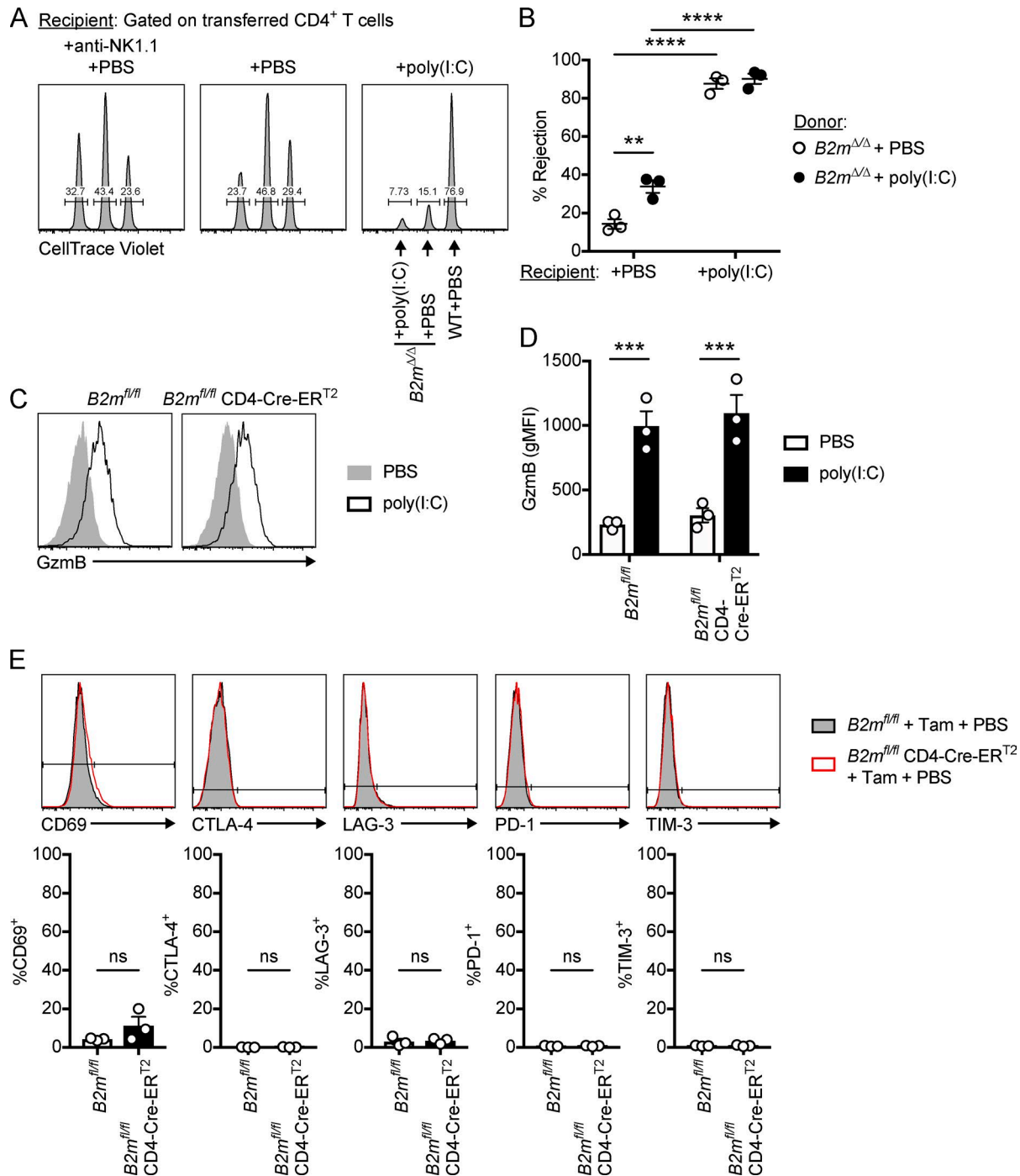


Figure S4. Poly(I:C) enhances NK cell cytotoxic function. (A and B) In vivo cytotoxicity assays were performed with donor splenocytes from WT and *B2m*^{ΔΔ} mice that were pretreated with PBS or 100 μg poly(I:C) 18 h previously. Bulk splenocytes were differentially labeled and injected i.v. into WT recipient mice that were pretreated with PBS or 100 μg poly(I:C) 24 h previously. Some recipients were depleted of NK cells by injecting anti-NK1.1 on days -2 and -1. **(A)** Representative histograms showing relative recovery after 3 h of transferred CD4⁺ T cells (CFSE⁺ CD19⁻ CD3⁺ CD4⁺) differentially labeled with CT violet. **(B)** NK cell-specific rejection of transferred *B2m*^{ΔΔ} CD4⁺ T cells after 3 h (data are combined from three independent experiments; two-way ANOVA with Bonferroni's multiple comparisons test). Each symbol in B represents the average of two to three recipient mice from an individual experiment that received the same mix of donor cells. **(C-E)** *B2m*^{fl/fl} and *B2m*^{fl/fl} CD4-Cre-ERT2 mice were treated with tamoxifen (Tam) on days 0-4 and injected i.p. with PBS or 100 μg poly(I:C) on day 6. Splenocytes were harvested 18 h after poly(I:C) treatment (day 7). **(C)** Representative histograms showing granzyme B expression in splenic NK cells (CD45⁺ CD19⁻ CD3⁻ NK1.1⁺ Nkp46⁺). **(D)** Geometric mean fluorescence intensity (gMFI) of granzyme B (GzmB) in NK cells (*n* = 3 mice per group; two-way ANOVA with Bonferroni's multiple comparisons test). **(E)** Representative histograms (top) with quantification (bottom) showing the percentage of NK cells that express the indicated marker (*n* = 3 mice per group; unpaired *t* test). Data in D and E are representative of two independent experiments with two to three mice per group per experiment. Each symbol in D and E represents an individual mouse. Error bars indicate mean ± SEM; **, *P* < 0.01; ***, *P* < 0.001; ****, *P* < 0.0001; ns, not significant.