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Supplemental information

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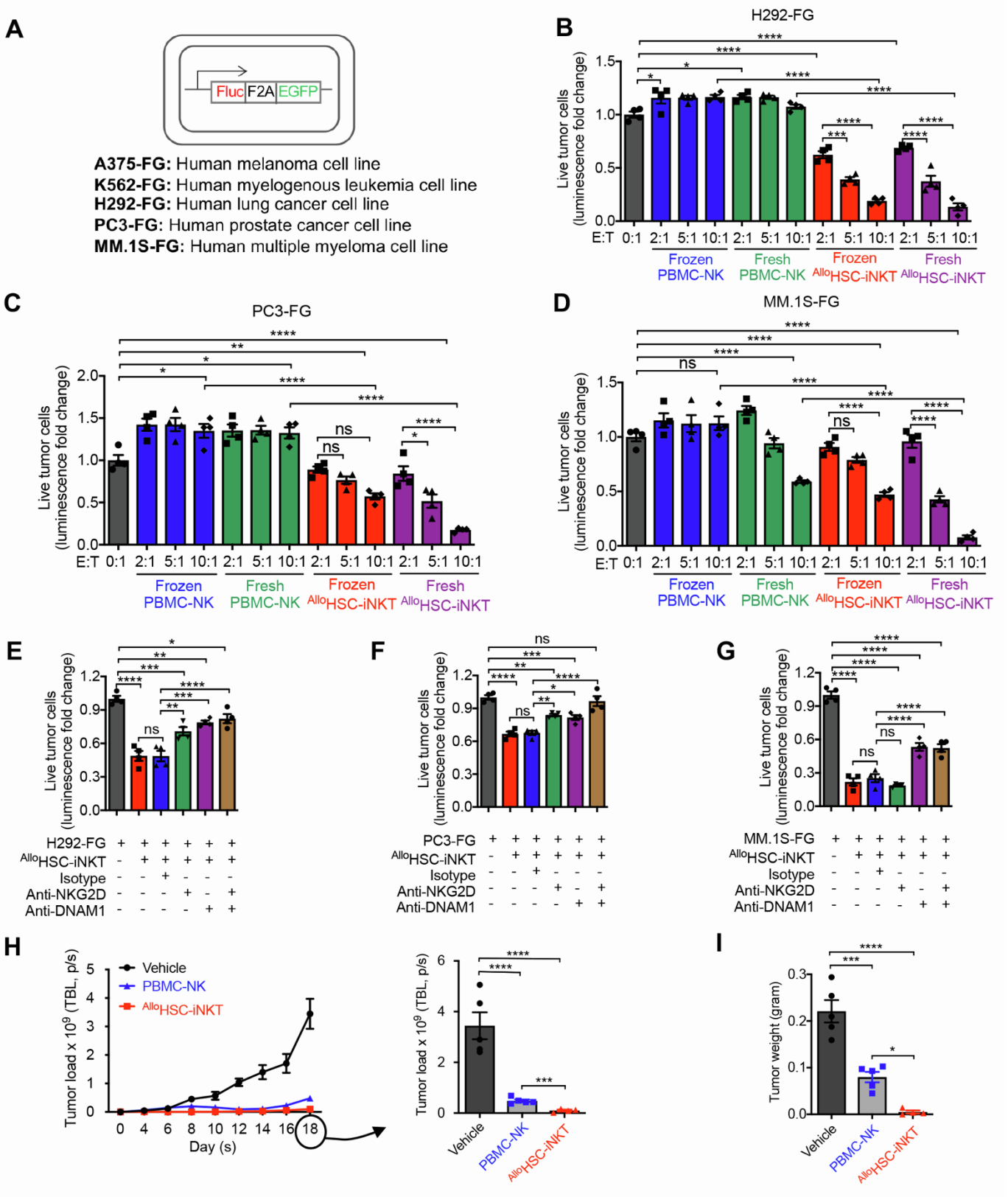

Figure S1. Tumor Targeting of ^{Allo}HSC-iNKT Cells Through Intrinsic NK Function; Related to Figure 3.

(A) Schematics showing the engineered A375-FG, K562-FG, H292-FG, PC3-FG, and MM.1S-FG cell lines. Fluc, firefly luciferase; EGFP, enhanced green fluorescent protein; F2A, foot-and-mouth disease virus 2A self-cleavage sequence.
(B-D) *In vitro* direct killing of human tumor cells by ^{Allo}HSC-iNKT cells. PBMC-NK cells were included as a control. Both fresh and frozen-thawed cells were studied. Tumor cell killing was analyzed at 24-hours post co-culture. Tumor killing data of H292-FG human lung cancer cells (B), PC3-FG human prostate cancer cells (C), and MM.1S-FG human multiple myeloma cells (D) were presented. N = 4. Related to main Figures 3C-3E.
(E-G) Tumor killing mechanisms of ^{Allo}HSC-iNKT cells. NKG2D and DNAM-1 mediated pathways were studied. Tumor cell killing was analyzed at 24-hours post co-culture. Tumor killing data of H292-FG (tumor:iNKT ratio 1:2), PC3-FG (tumor:iNKT ratio 1:10), and MM.1S-FG (tumor:iNKT ratio 1:15) were presented. N = 4. Related to main Figures 3F-3H.
(H-I) *In vivo* antitumor efficacy of ^{Allo}HSC-iNKT cells in an A375-FG human melanoma xenograft NSG mouse model. (H) BLI measurements of tumor loads over time (n = 4 or 5). (I) Tumor weight at the terminal harvest on day 18 (n = 4 or 5). Related to main Figures 3I-3K.
Representative of 3 experiments. Data are presented as the mean ± SEM. ns, not significant, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, by 1-way ANOVA (B-G, I) or by Student's *t* test (H).

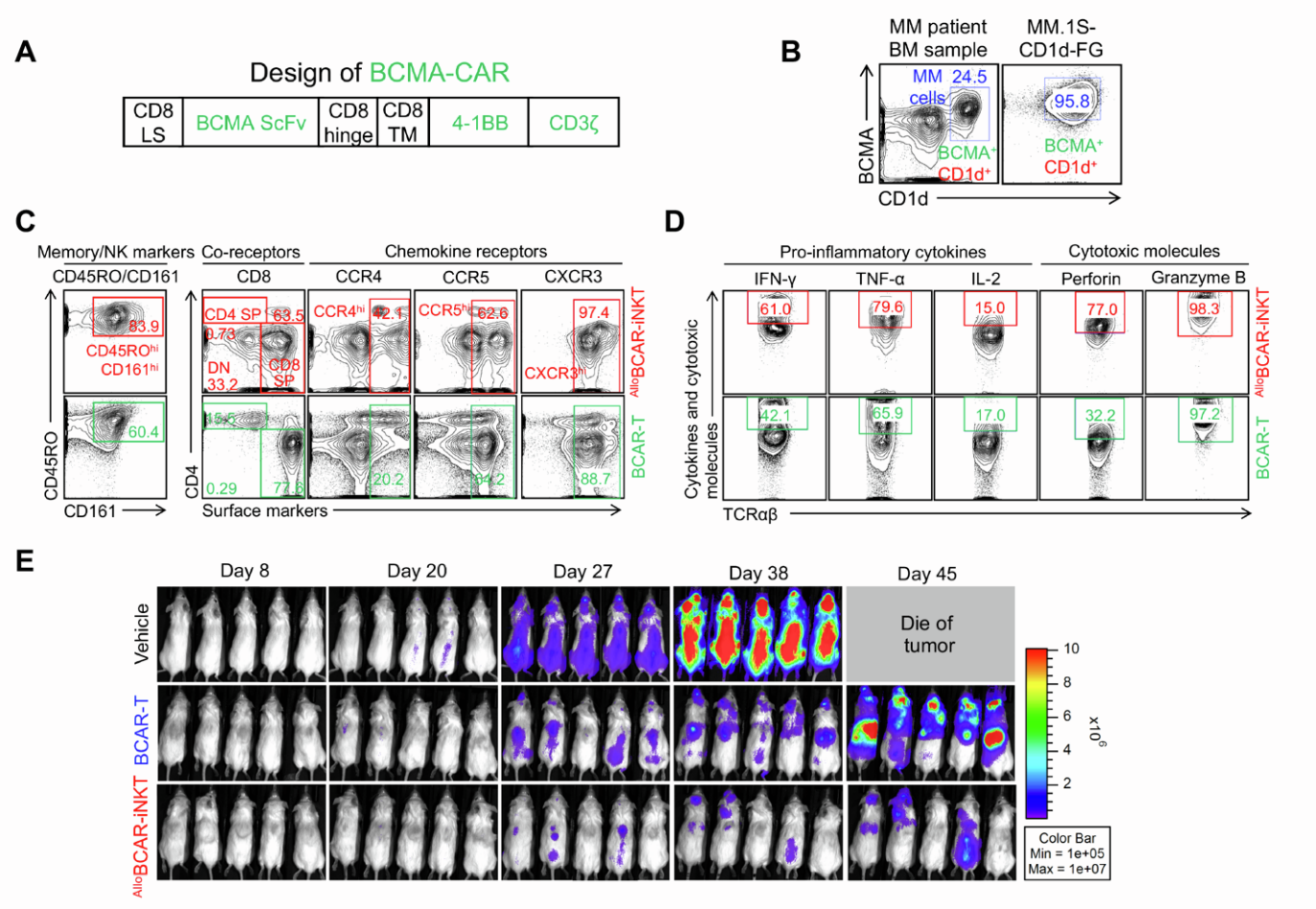

Figure S2. Tumor Targeting of ^{Allo}HSC-iNKT Cells Through Engineered Chimeric Antigen Receptors; Related to Figure 4.

(A) Schematics showing the design of BCMA-CAR. LS, leader sequence; ScFv, single-chain variable fragment; TM, transmembrane domain.

(B) FACS analyses of BCMA and CD1d expression on MM.1S-CD1d-FG cells. A representative MM patient primary bone marrow (BM) sample was included as a control.

(C-D) FACS characterization of ^{Allo}BCAR-iNKT cells. (C) Surface marker expression. (D) Intracellular cytokine and cytotoxic molecule production. BCAR-T cells were included as a control.

(E) *In vivo* antitumor efficacy of ^{Allo}BCAR-iNKT cells in an MM.1S-CD1d-FG human multiple myeloma xenograft NSG mouse model. BLI images were presented showing tumor loads in experimental mice over time. Related to main Figures 4K-4N.

Representative of 2 (E) and 3 (A-D) experiments.

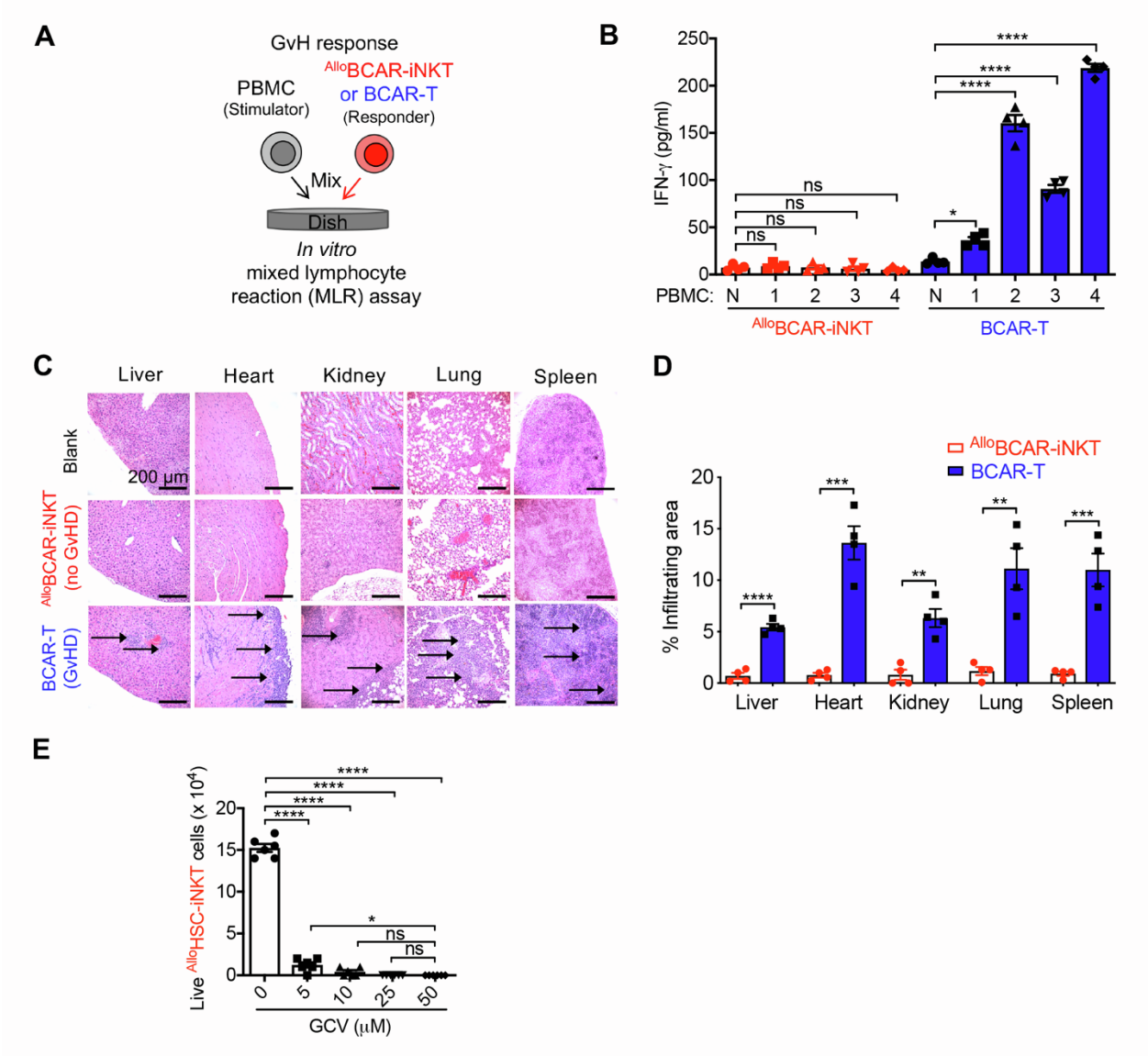

Figure S3. Safety study of ^{Allo}HSC-iNKT cells; Related to Figure 5.

(A-B) Studying the graft-verus-host (GvH) response of ^{Allo}BCAR-iNKT cells using an *in vitro* mixed lymphocyte reaction (MLR) assay. BCAR-T cells were included as a responder cell control. (A) Experimental design. PBMCs from 4 different healthy donors were used as stimulator cells. (B) ELISA analyses of IFN-γ production at day 4 (n = 4).

(C-D) Histology analysis of tissue sections collected from experimental mice as described in Figures 4G-4J. (C) H&E-stained tissue sections. Blank indicates tissue sections collected from NSG mice receiving no adoptive cell transfer. Arrows point to mononuclear cell infiltrates. Scale bar: 200 μm. (D) Quantification of C (n = 4).

(E) *In vitro* GCV killing assay. ^{Allo}HSC-iNKT cells were cultured *in vitro* in the presence of gradient concentrations of GCV for 4 days, followed by quantification of live cells via cell counting (n = 6).

Representative of 2 experiments. Data are presented as the mean ± SEM. ns, not significant, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, by Student's *t* test (D) or by 1-way ANOVA (B and E).

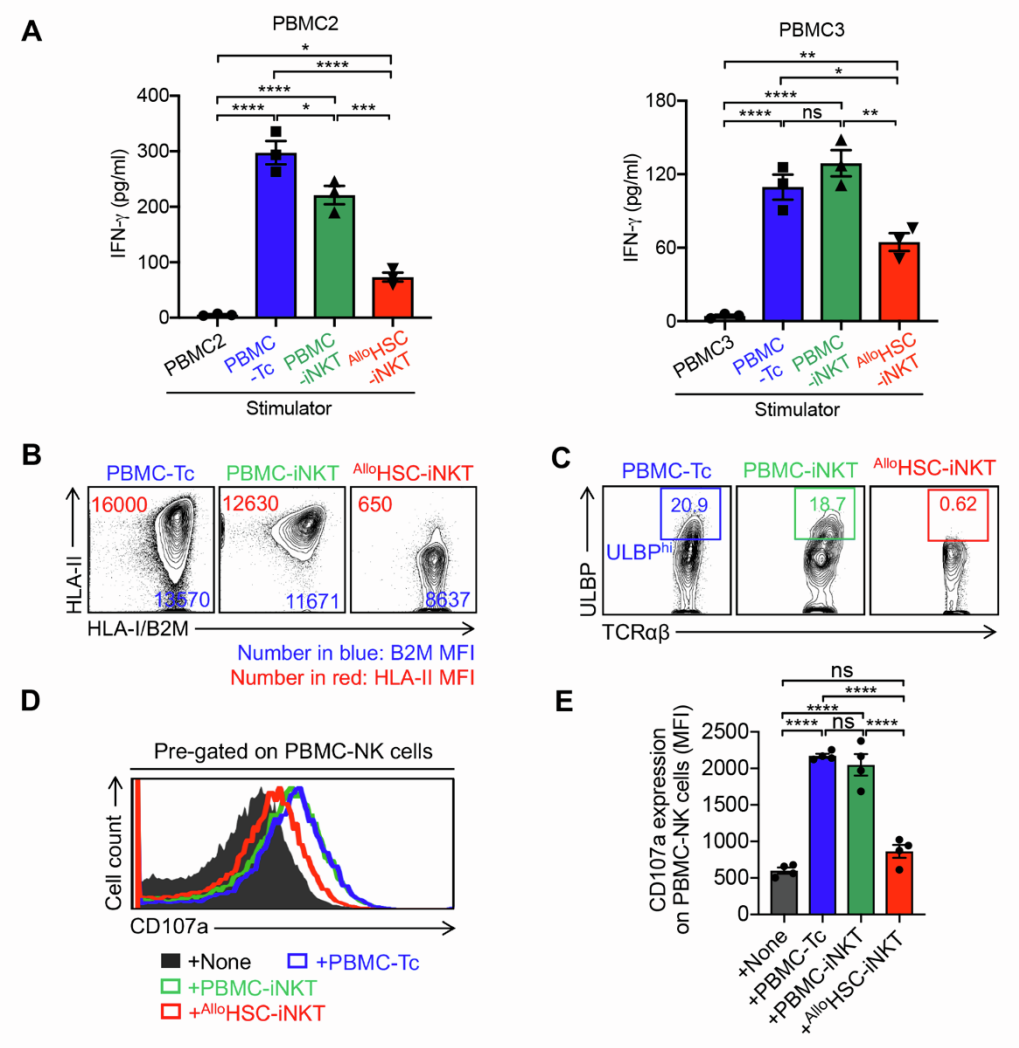

Figure S4. Immunogenicity Study of ^{Allo}HSC-iNKT Cells; Related to Figure 6.

(A) ELISA analyses of IFN-γ production at day 4 in an *in vitro* MLR assay (n = 3). Data of PBMC2 and PBMC3 responders were presented. Related to main Figures 6A and 6C.

(B) FACS analyses of HLA-I/II expression on PBMC-Tc, PBMC-iNKT, and ^{Allo}HSC-iNKT cells. Representative FACS plots were presented. Related to main Figure 6B.

(C) FACS analyses of ULBP expression on PBMC-Tc, PBMC-iNKT, and ^{Allo}HSC-iNKT cells. Representative FACS plots were presented. Related to main Figure 6F.

(D-E) Studying CD107a expressions on PBMC-NK cells using an *in vitro* MLR assay. PBMC-NK cells were co-cultured with ^{Allo}HSC-iNKT, PBMC-iNKT, and PBMC-Tc cells for 24 hours. CD107a antibody was added into the cell cultures and incubated for 2 hours prior to flow cytometry. (D) FACS analyses of CD107a expression on PBMC-NK cells. (E) Quantification of D (n = 4).

Representative of 3 experiments. Data are presented as the mean ± SEM. ns, not significant, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, by one-way ANOVA.

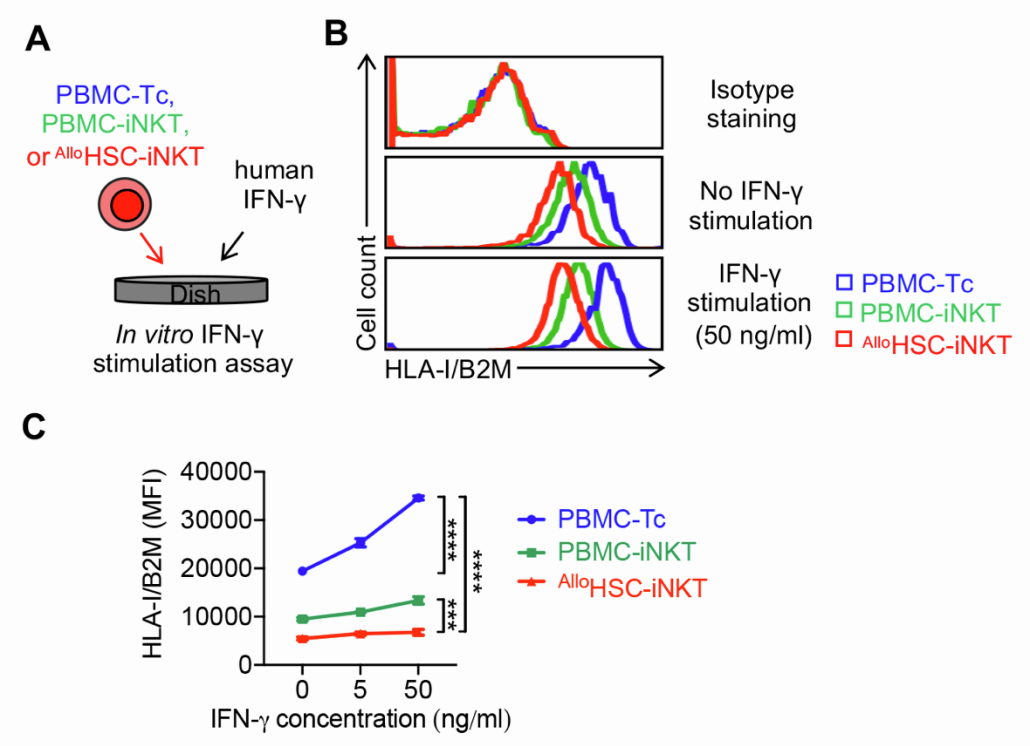

Figure S5. HLA-I Expression Study on ^{Allo}HSC-iNKT Cells Under IFN-γ Stimulation; Related to Figure 6.

(A) Experimental design. ^{Allo}HSC-iNKT cells were stimulated with a range of IFN-γ (0, 5, and 50 ng/ml) for 3 days. PBMC-iNKT and PBMC-Tc cells were included as controls.

(B) FACS analyses of HLA-I expression on the indicated cells.

(C) Quantification of HLA-I expression on the indicated cells stimulated with IFN-γ of indicated concentrations (n = 3).

Representative of 3 experiments. Data are presented as the mean ± SEM. *P < 0.05, ***P < 0.001, ****P < 0.0001, by 1-way ANOVA.

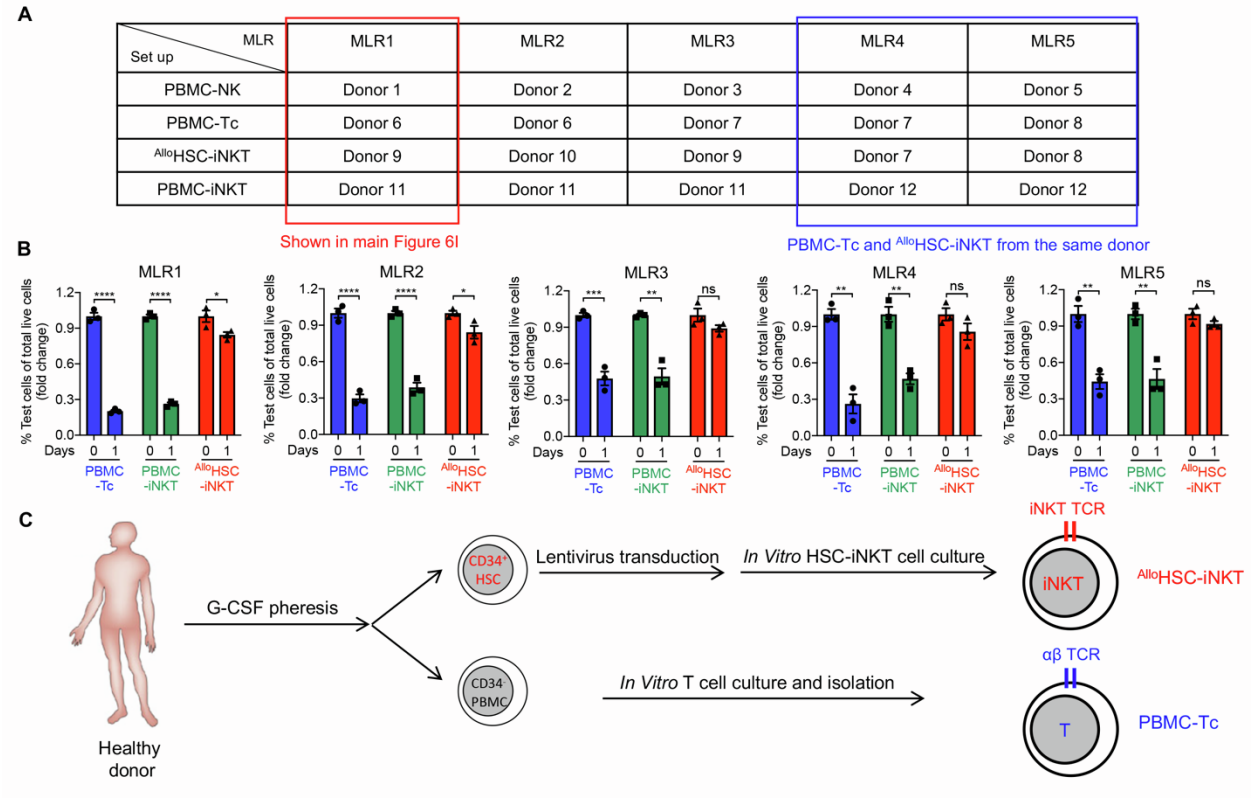

Figure S6. Studying NK cell-mediated allorejection of ^{Allo}HSC-iNKT cells; Related to Figure 6.

(A) Table showing the donor information of MLR assay. Five different sets of MLR assay were performed; three sets used PBMC-Tc and ^{Allo}HSC-iNKT cells from different donors, two sets used PBMC-Tc and ^{Allo}HSC-iNKT cells from the same donors. Data of MLR1 have been shown in Figure 6I.

(B) Quantification of the indicated test cells (n = 3).

(C) Diagram showing the experimental procedure to generate PBMC-Tc and ^{Allo}HSC-iNKT cells from the same donor. Data are presented as the mean ± SEM. ns, not significant, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, by Student's *t* test.

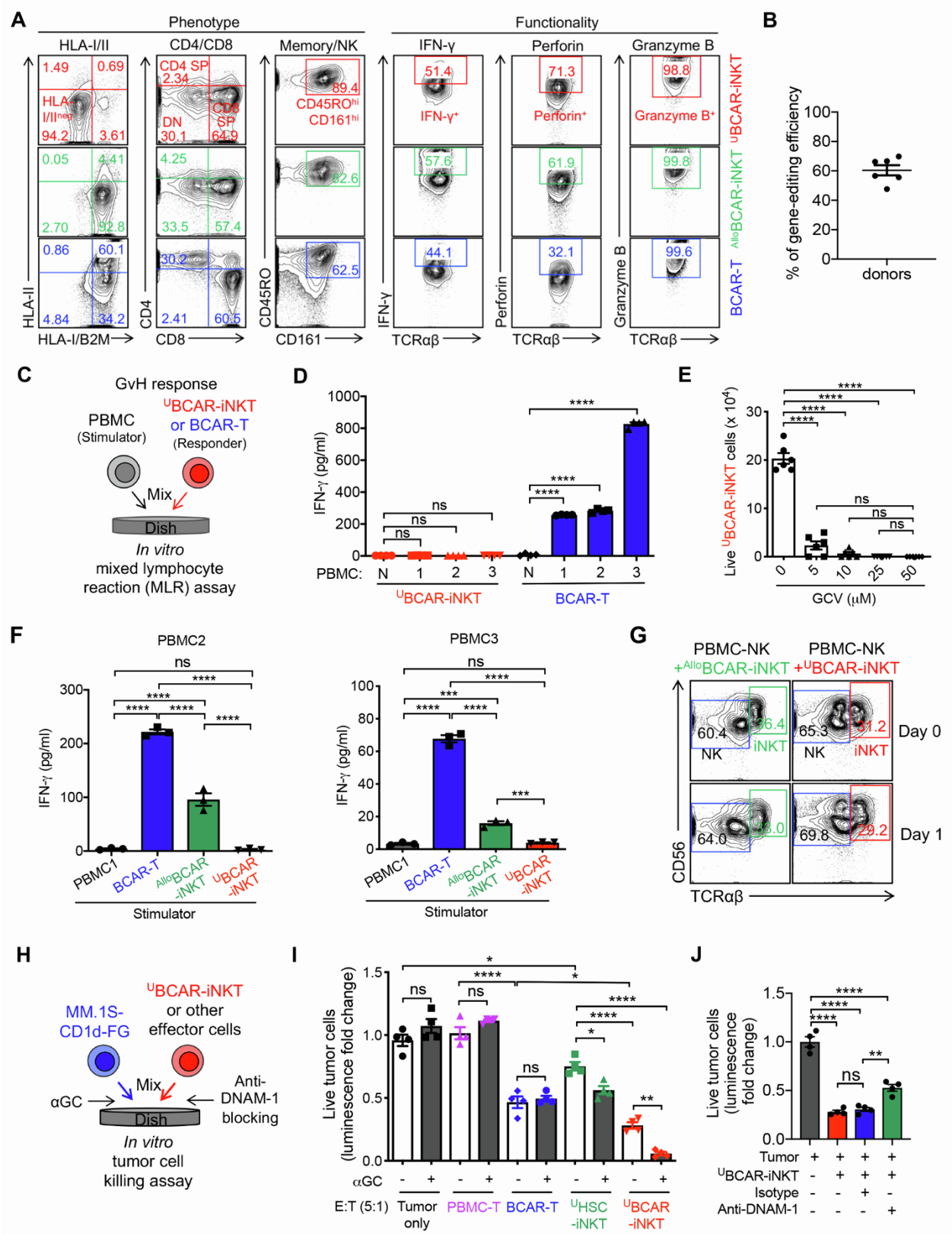

Figure S7. Development of HLA-Ablated Universal HSC-iNKT ([U]HSC-iNKT) Cells and Derivatives; Related to Figure 7.

(A) FACS analyses of surface marker expression, and Intracellular cytokine and cytotoxic molecule production by ^{U}BCAR-iNKT cells. ^{Allo}BCAR-iNKT and BCAR-T cells were included as controls.
(B) The genome editing efficiencies among multiple HSC donors.
(C-D) Studying the GvH response of ^{u}BCAR-iNKT cells using an *in vitro* MLR assay. BCAR-T cells were included as a responder cell control. (C) Experimental design. PBMCs from 3 different healthy donors were used as stimulator cells. (D) ELISA analyses of IFN-γ production at day 4 (n = 4).
(E) *In vitro* GCV killing assay. ^{U}BCAR-iNKT cells were cultured *in vitro* in the presence of gradient concentrations of GCV for 4 days, followed by quantification of live cells via cell counting (n = 6).
(F) Studying allogenic T cell response against ^{U}BCAR-iNKT cells using an *in vitro* MLR assay. ELISA analyses of IFN-γ production at day 4 were presented (n = 3). Related to main Figures 7E and 7F.
(G) Studying allogenic NK cell response against ^{U}BCAR-iNKT cells using an *in vitro* MLR assay. ^{Allo}BCAR-iNKT cells were included as a control. Representative FACS plots were presented, showing the quantification of the indicated cells at day 0 and day 1. Related to main Figures 7G and 7H.
(H-J) *In vitro* killing of MM.1S-CD1d-FG human multiple myeloma cells by ^{U}BCAR-iNKT cells. PBMC-T, BCAR-T, and ^{U}HSC-iNKT cells were included as effector cell controls. (H) Experimental design. (I) Tumor cell killing by the indicated effector cells with/out the addition of αGC (n = 4). (J) Tumor cell killing by ^{Allo}BCAR-iNKT cells with/out the blockade of DNAM-1 (n = 4). Tumor cell killing was analyzed at 8-hours post co-culture (effector:tumor ratio 5:1).
Representative of 3 experiments. Data are presented as the mean ± SEM. ns, not significant, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, by 1-way ANOVA.