

Overcoming host histocompatibility barrier to create a renewable source of Off-the-shelf effector lymphocytes for adoptive immunotherapy

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ABSTRACT

Encouraging clinical outcomes in autologous cellular immunotherapy have garnered hope and excitement. However, limitations of patient-derived cancer immunotherapies remain to be addressed to deliver reliable and efficacious therapies with broader applicability. Induced pluripotent stem cells (iPSCs) are a unique, renewable source for the continuous generation of cellular therapeutics and represent a highly promising approach for overcoming many of the limitations of autologous therapy. To advance the promise of iPSC technology as an “off-the-shelf” (OTS) source of cellular therapeutics, several considerations need to be addressed. Ensuring the persistence of allogeneic OTS therapies after adoptive cell transfer across histocompatibility barriers is a key requirement. Establishing a master cell line from genetically engineered clonal iPSC lines with the capacity to continuously generate homogenous populations of highly functional effector cells will also be necessary.

Here we demonstrate a comprehensive approach for the generation of immune tolerant effector cells derived from a genetically engineered iPSC master cell line. We successfully combined deletion of classical human leukocyte antigen molecules with expression of immunosuppressive proteins to generate clonal iPSC lines with the ability to escape immune rejection. Utilizing *in vitro* quantitative live cell analysis we show that OTS-iPSCs elicit a significantly decreased cytotoxic response from both peripheral blood (PB)-NK cells (47.9 vs. 91.4% survival at 3:1 E:T ratio) and PB-T cells (>2.7-fold greater number of OTS-iPSC derived cells remaining at 88 hrs). Additionally, mixed lymphocyte reactions employing unfractionated PB mononuclear cells resulted in significantly decreased activation and proliferation of CD8+ T cells (63.4 vs. 29.6%), CD4+ T cells (70.9 vs. 17.3%) and NK cells (46.8 vs. 11.6%). In preclinical mouse models we demonstrate that OTS-iPSCs exhibit improved persistence *in vivo*. Bilateral engraftments were established in non-conditioned, fully immune-competent recipient mice using luciferized wildtype and OTS-iPSCs. Daily bioluminescence imaging revealed a significant increase in persistence of OTS-iPSCs during the 48-196 hour post injection window (>5.5 fold greater luminescence at 96 hrs). Using our potent chemically-defined stage-specific monolayer hematopoietic differentiation platform, we demonstrate that OTS-iPSC derived CD34 expressing hematopoietic cells are reproducibly scaled and readily give rise to functional lymphocytes carrying the engineered targeted modality in a homogenous manner (95 +/- 5%). The outlined preclinical data illustrate that iPSCs are an ideal renewable source for OTS hematopoietic cell-based immunotherapies and represent a potentially exponential advancement in adoptive immunotherapy.

SUMMARY

- ❖ It is possible to extensively and accurately engineer multiple modalities into a single pluripotent stem cell to generate a master cell line for off-the-shelf immunotherapeutics
- ❖ Increased persistence and immune evasion can be achieved via HLA class I modifications
- ❖ Overexpression of immunosuppressive proteins can further improve the persistence of hiPSC-derived effector cells
- ❖ Engineered clonal hiPSC lines can be differentiated into effector cell types such as T lymphocytes that can be matured and expanded *in vitro* for the generation of histocompatible off-the-shelf therapeutics

REFERENCES

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RESULTS

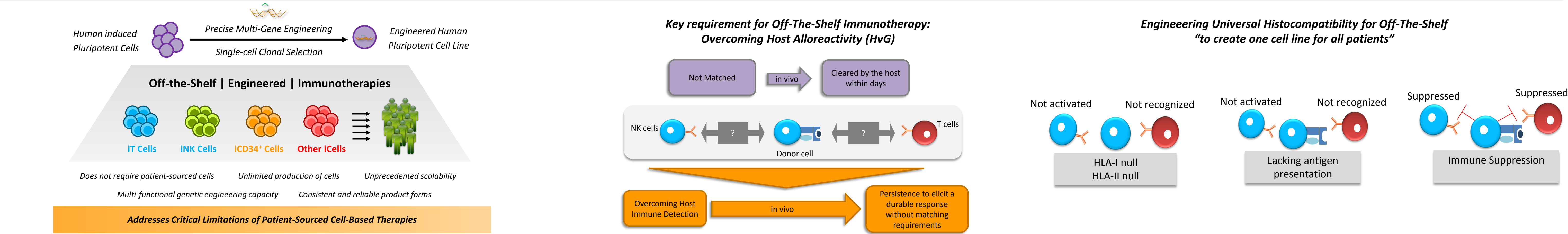


Figure 1. Schematic representation of pluripotent cell platform for off-the-shelf adoptive immunotherapy. Human Induced pluripotent stem cells represent a unlimited source of immune effector cells for cell based therapies. To create a master cell line that has universal capacity for all patients the key challenge of overcoming host alloreactivity needs to be addressed. Our platform for the generation of precisely engineered hiPSC with multiple immune evasion and immune suppression modalities represents an optimal platform for universal off-the-shelf immunotherapy.

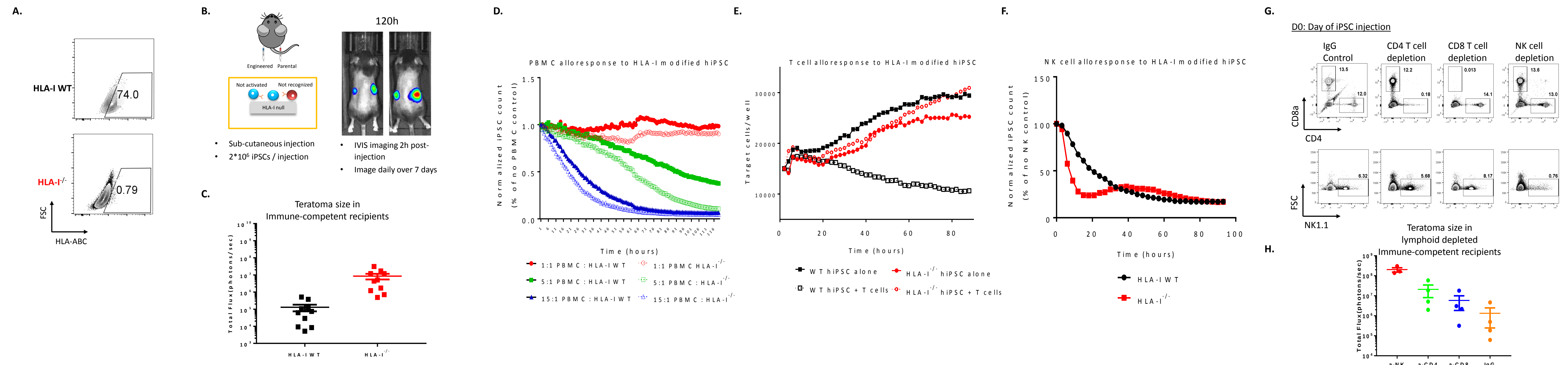


Figure 2. Universal histocompatibility and enhanced persistence through HLA-I modification of hiPSC. A. Several genetic engineering strategies were employed to generate HLA-I hiPSCs with mid, low and negative HLA-I cell surface expression. Phenotypic analysis by flow cytometry of HLA-engineered hiPSC for HLA class I expression. B. Schematic description of bilateral teratoma study design. C. Quantification and representative bioluminescence imaging of C57BL/6 mice transplanted with the indicated hiPSCs 120 hours after transplantation. Date demonstrates that HLA-I^{-/-} hiPSC display the highest increase in persistence D. Immune recognition and cytotoxicity of activated peripheral blood-derived NK cells against HLA-I^{-/-} hiPSC was assessed over a 4 day culture assay using fluorescent live cell imaging. Cytotoxicity was assessed by determining the number of live hiPSC remaining in the culture compared to those cultured without the addition of PBMCs. E. HLA-I^{-/-} hiPSC are more rapidly detected and eliminated by PBMC at increasing effector:target ratios. E. Immune recognition and cytotoxicity of peripheral blood T cells against HLA-engineered hiPSC was assessed over a 4 day culture assay using fluorescent live cell imaging. HLA-I^{-/-} hiPSC are more rapidly detected and destroyed by NK cells demonstrating the need for further immune evasion strategies. G. To confirm that NK cells are the components of the immune system were responsible for the rapid detection and elimination of the hiPSCs teratoma assays were performed in immune-competent C57BL/6 recipient mice that were previously treated with anti-CD4, anti-CD8, anti-NK1.1 or IgG control antibodies for 3 days to deplete the T and NK cell subsets. Flow cytometry analysis of peripheral blood depicting the efficient deletion of CD4+ T cells, CD8+ T cells and NK1.1+ NK cells from recipient mice. H. Quantification of C57BL/6 mice with the HLA-I WT hiPSCs 5 days after transplantation demonstrates that the depletion of the NK cell subset most effectively increased the persistence of HLA-I WT hiPSCs.

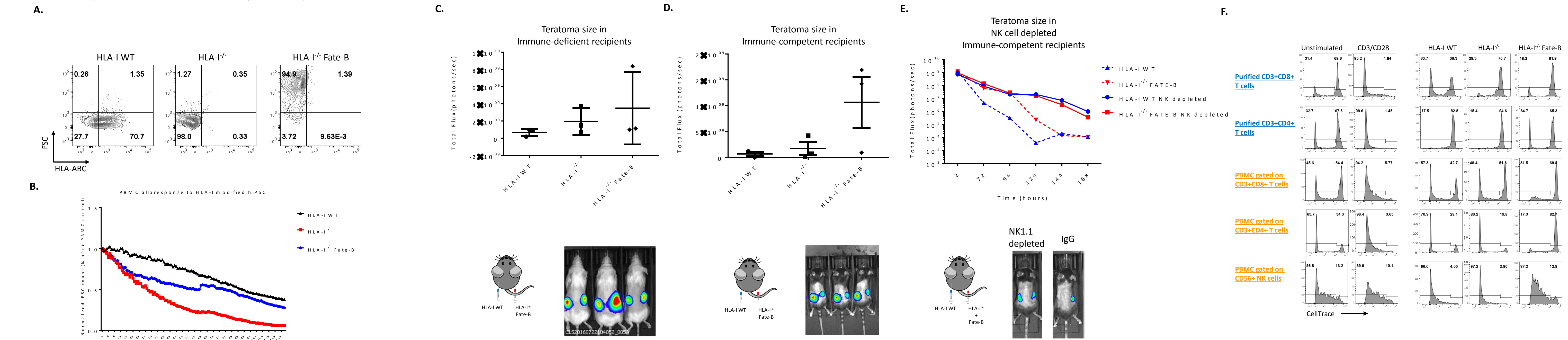


Figure 3. Multilayer genetic engineering of HLA-I^{-/-} hiPSC with immunosuppressive modalities increases persistence and immune evasion in vivo. A. Phenotypic analysis by flow cytometry of HLA-engineered hiPSC for immune suppression molecule Fate-B. B. Immune recognition and cytotoxicity of fresh PB-derived NK cells against engineered HLA-I^{-/-} hiPSC was assessed over a 4 day culture assay using fluorescent live cell imaging. Cytotoxicity was assessed by determining the number of live hiPSC remaining in the culture compared to those cultured without the addition of NK cells. C. Persistence and immune evasion of engineered HLA-I^{-/-} hiPSC was assessed using bilateral teratoma assay in immunocompromised NSG recipient mice. Quantification and representative bioluminescence imaging of NSG mice transplanted with the indicated hiPSCs 96 hours after transplantation D. Persistence and immune evasion of HLA-I^{-/-} engineered hiPSC was assessed by bilateral teratoma assay in immune-competent C57BL/6 recipients. Quantification and representative bioluminescence imaging of C57BL/6 mice with the indicated hiPSCs 96 hours after transplantation demonstrates that Fate-B modality increases persistence of HLA-I^{-/-} hiPSC. E. Quantification and representative bioluminescence imaging of NK cell depleted C57BL/6 mice with the HLA-I WT and HLA-I^{-/-} Fate-B hiPSCs demonstrates that additional NK immunosuppressive strategies need to be considered to fully suppress the NK cell recognition and cytotoxicity of engineered hiPSCs. F. Engineered HLA-I^{-/-} hiPSCs demonstrate increased immune suppression and persistence in vitro. To assess the ability of the engineered HLA-I^{-/-} hiPSCs to induce an alloreactive immune response a modified mixed lymphocyte reaction (MLR) was performed. Purified peripheral blood (PB) T cells or whole peripheral blood mononuclear cells (PBMCs) were labeled with cell trace violet to monitor cellular proliferation and placed in culture with HLA-I^{-/-} hiPSCs for 11 days in the presence of IL2. T cells cultured alone with or without CD3/CD28 bead stimulation served as negative and positive controls respectively. Flow cytometry analysis of cell trace violet within CD8+ and CD4+ gated CD3+ T cells and CD3-CD56+ NK cells is shown.

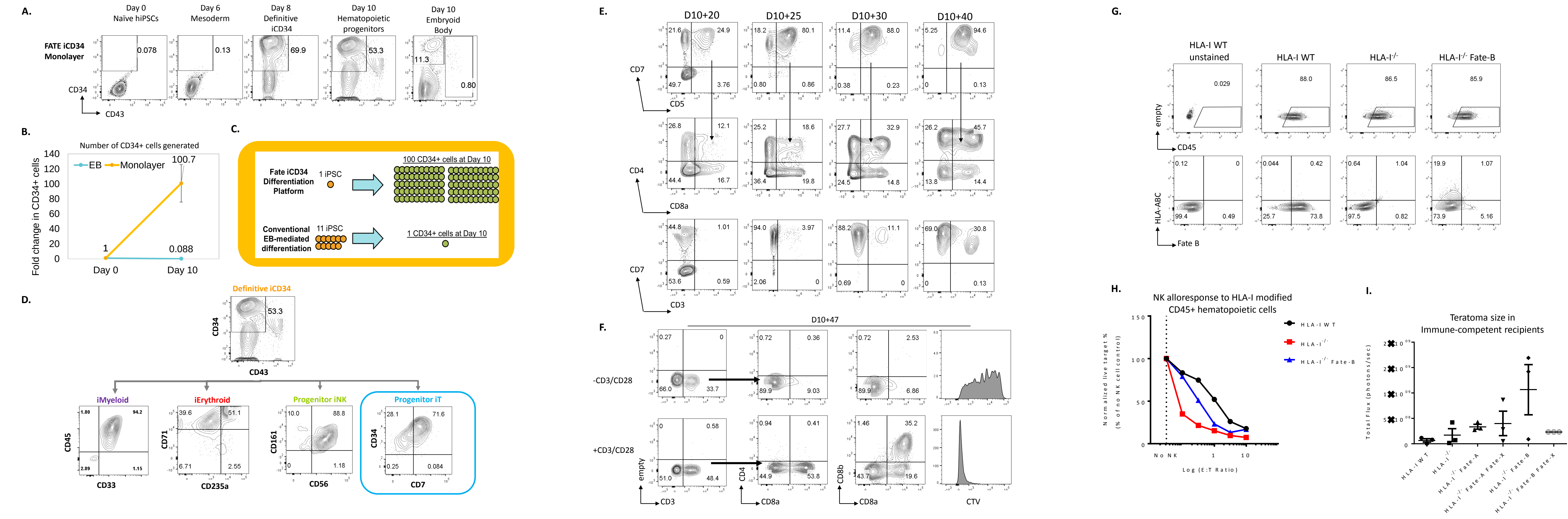


Figure 5. HLA-I^{-/-} Fate-B hiPSCs can differentiate into lymphoid effector cells and maintain expression of engineered modalities. A. Multi-staged, defined, feeder free, monolayer culture process for the hematopoietic differentiation of naive hiPSCs to CD34+ hemogenic endothelium (HE, iCD34). Monolayer iCD34 platform generates more CD34+ HE than conventional Embryoid body(EB)-based protocols. hiPSCs were aggregated to form EBs or seeded as a monolayer for 10 days prior to analysis for CD34 and CD43 expression. B. CD34 positive cells were counted and plotted over time for monolayer and EB mediated hematopoietic differentiation. C. Robust scalability of the iCD34 differentiation platform as illustrated by the number of input iPSCs required to generate CD34+ HE. D. Day 10 iCD34 HE was isolated and placed in differentiation assays for myeloid, erythroid, NK lymphoid and T lymphoid cells. E. After 7 days of myeloid differentiation cultures were assessed for monocyte and macrophage markers CD45 and CD33. F. After 7 days of erythroid differentiation cultures were assessed for the expression of CD71 and CD235a. G. After 10 days of T lymphoid differentiation cultures were assessed for the expression of CD3 and CD7 to identify T lymphoid progenitors. H. After 10 days of NK lymphoid differentiation cultures were assessed for the expression of CD56 and CD161 to identify NK progenitors. I. Generation of functional T lymphocytes from numerous sources of engineered hiPSCs. J. Day 10 iCD34 cells from fibroblast-derived hiPSCs were differentiated into iT cells and assessed at the indicated timepoints for the expression of T cell maturation markers. K. Day 10 iCD34 cells were assessed for their functional capacity to respond to CD3/CD28 stimulation. L. iT cells were labeled with cell trace violet to monitor cellular proliferation and left unstimulated or stimulated with CD3/CD28 dynabeads for 7 days. M. D10-47 iT cells were assessed by flow cytometry and found to proliferate and differentiate into CD8+ effector T cells in response to stimulation. N. Engineered HLA-I^{-/-} Fate-B iPSC can differentiate into CD45+ lymphoid cells and maintain integrity of engineered modalities. O. Indicated hiPSC lines were differentiated to CD34 HE and then subsequently into CD45+ lymphoid progenitors as described in D. Phenotypic analysis by flow cytometry demonstrates the maintenance of HLA-I deletion and Fate-B protein expression. P. HLA-I^{-/-} Fate-B-derived lymphoid cells maintain the ability to suppress NK cell mediated recognition and cytotoxicity. Q. Immune recognition and cytotoxicity of fresh PB-derived NK cells against engineered HLA-I^{-/-} Fate-B derived CD45+ lymphoid cells was assessed over a 4 day culture assay using fluorescent live cell imaging. R. Cytotoxicity was assessed by determining the number of live hiPSC remaining in the culture compared to those cultured without the addition of NK cells. S. Persistence and immune evasion of additional combinatorial strategies for HLA-I^{-/-} engineered hiPSC was assessed by bilateral teratoma assay in immune-competent C57BL/6 recipients.