Society for Immunotherapy of Cancer

GENERATION OF MATURE IPSC-DERIVED CAR T CELLS WITH ENHANCED ANTITUMOR ACTIVITY VIA EPIGENETIC REPROGRAMMING

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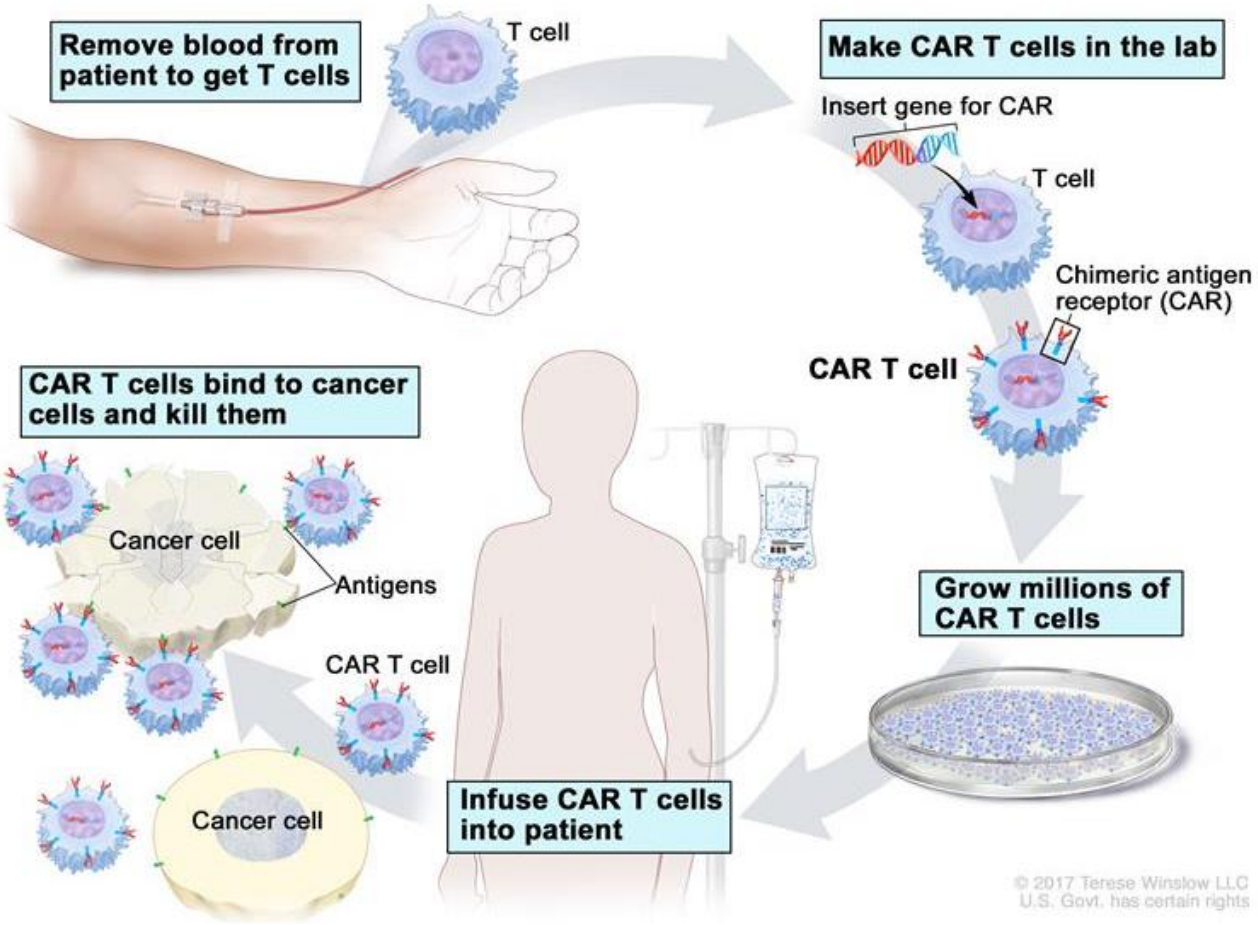
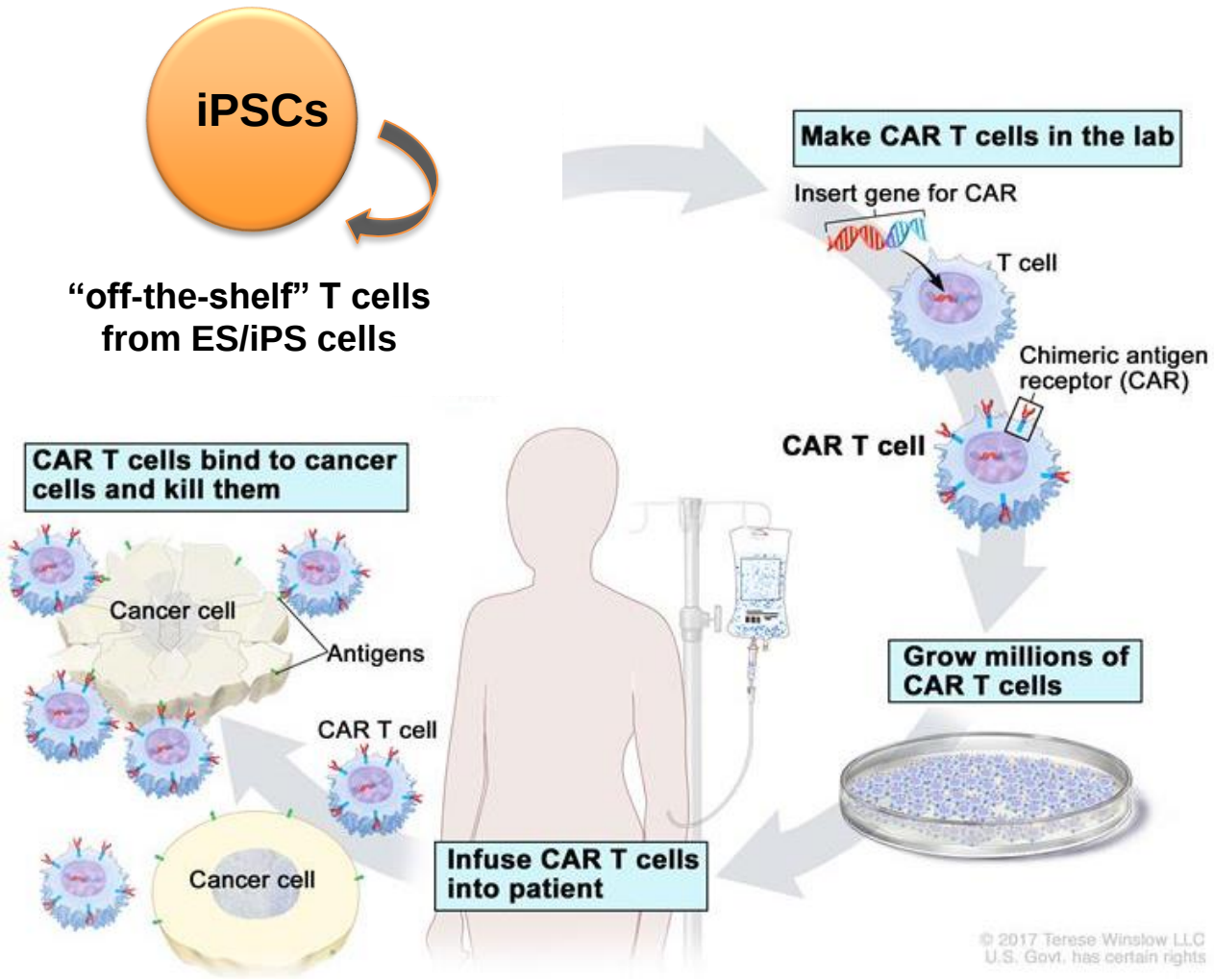
Current CAR T cell therapy



Autologous T cells:

- No graft rejection
- No graft-vesus-host disease (GVHD)
- Impaired Starting material
- Heterogeneous drug product
- Complex logistic
- Time consuming/Expensive

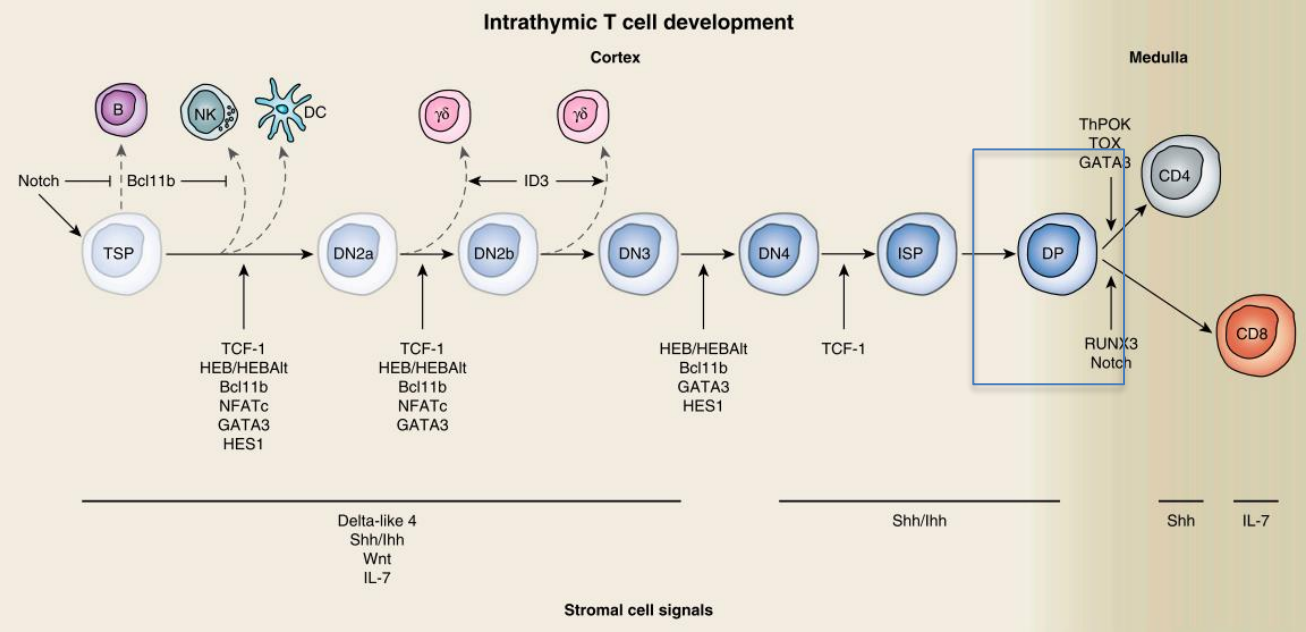
Human pluripotent stem cells for CAR T cell therapy



iPSC-T cells:

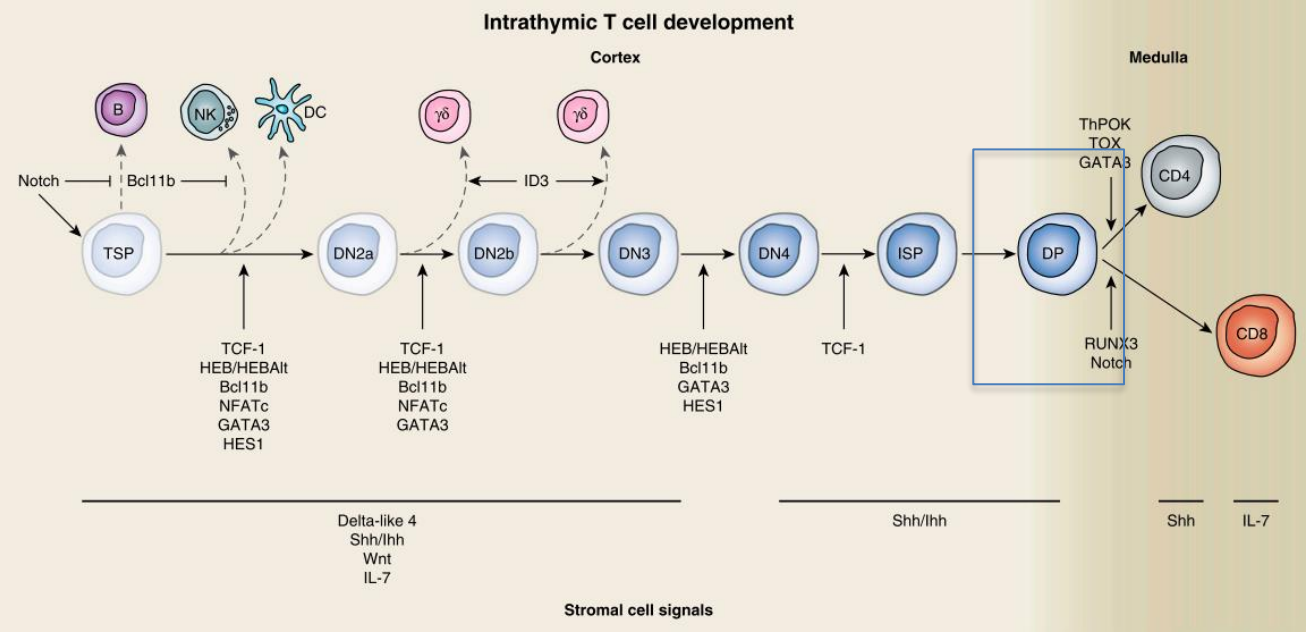
- Health Starting material
- Homogeneous drug product
- Simple logistic
- Cost-effective
- Efficient differentiation into mature T cells?

Generation of T cells from iPSCs



Shah *et al,* 2014

Generation of T cells from iPSCs



Shah *et al,* 2014

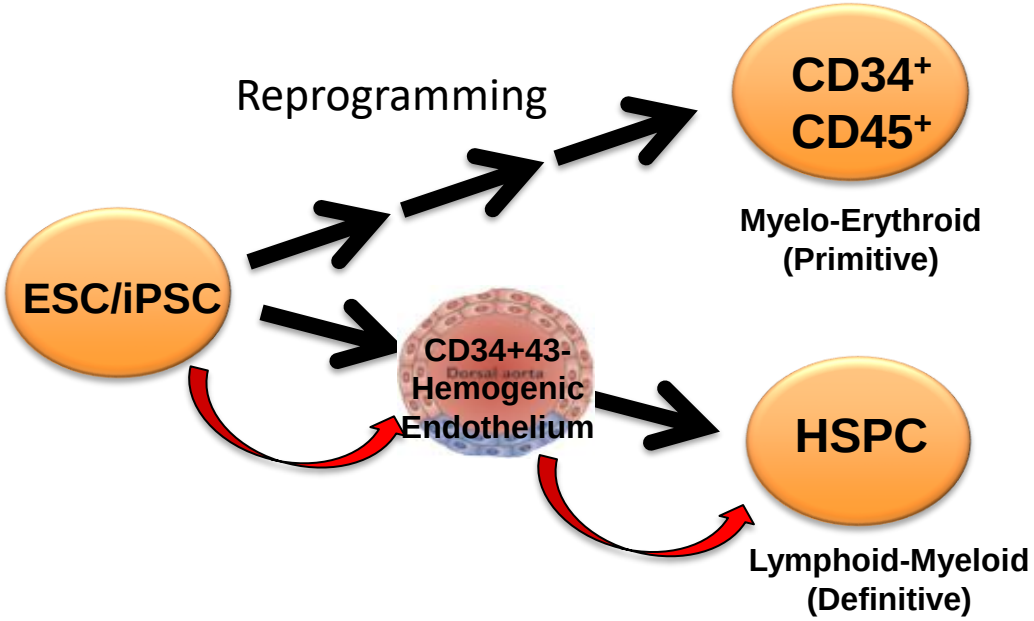
- Co-culture with mouse stromal cells (OP9-DLL1/DLL4)
- Cytokines: IL-7. SCF, Flt3

  Low efficiency

  T cells with innate (γδ-like) phenotypes (Themeli et al., 2013)

- 3D organoid (Vizcardo et al., 2018, Montel-Hagen et al., 2019)
- Stroma-free (Shukla et al., 2017, Iriguchi et al., 2021)

Identification of new regulators for T cell differentiation



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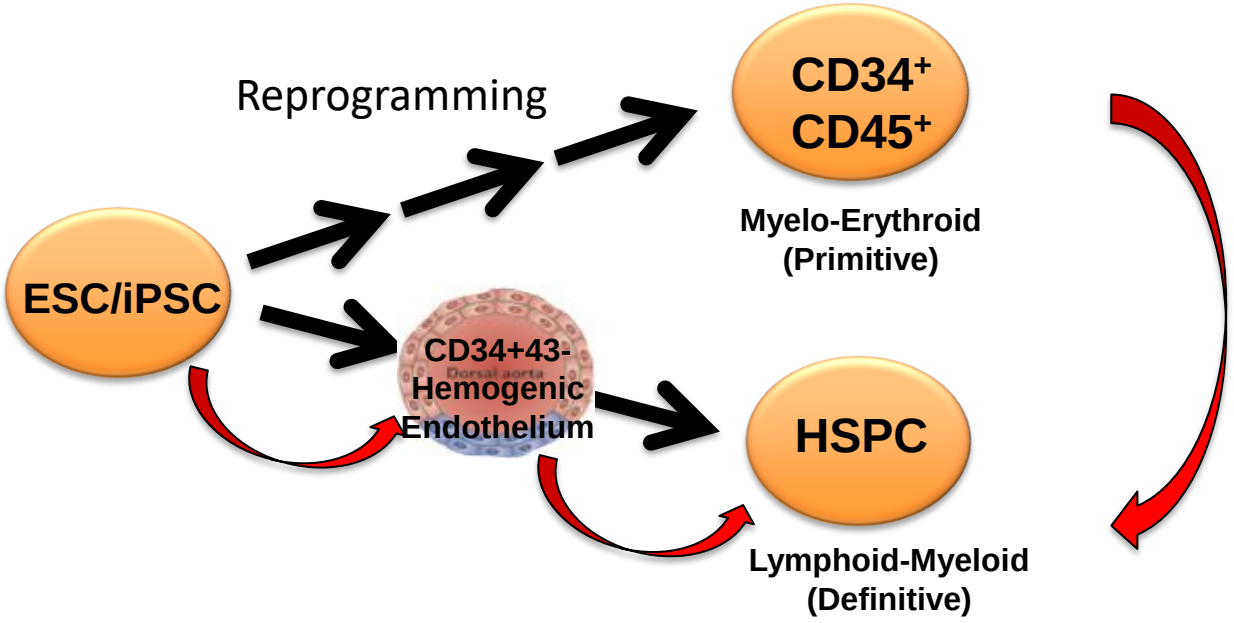
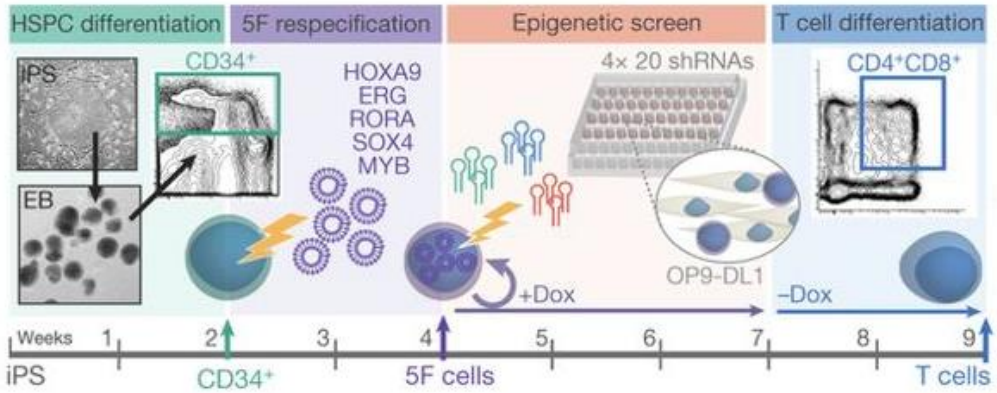


Screen for
Epigenetic Factors that
regulate Multipotency

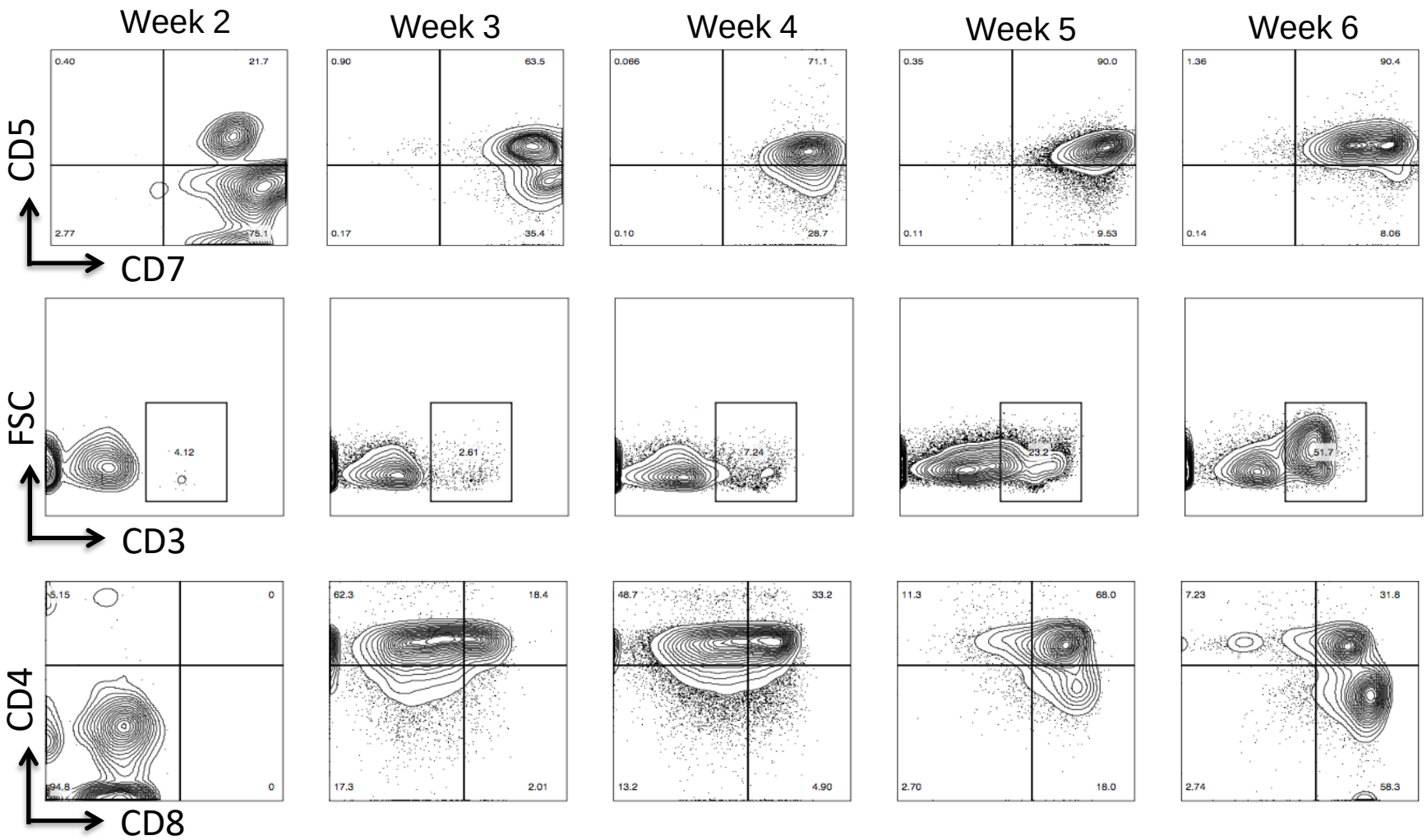
Nature. 2018 Jan 25;553(7689):506-510. doi: 10.1038/nature25435. Epub 2018 Jan 17.

Regulation of embryonic haematopoietic multipotency by EZH1.

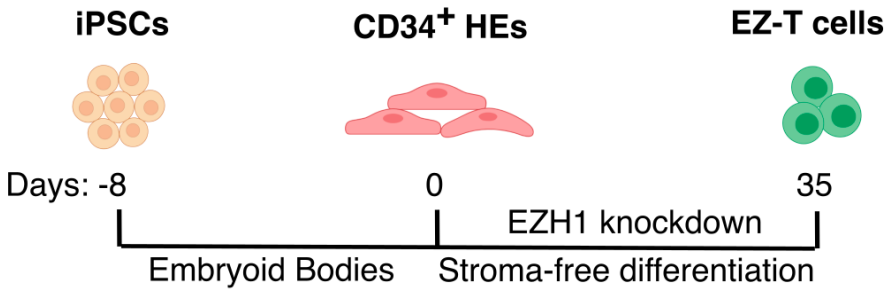
Vo LT[1,2,3], Kinney MA[1,2], Liu X[4], Zhang Y[4,5], Barragan J[1,2], Sousa PM[1,2], Jha DK[1,2], Han A[1,2], Cesana M[1,2], Shao Z[5], North TE[6], Orkin SH[2,3,7], Doulatov S[8], Xu J[4], Daley GQ[1,2,3].

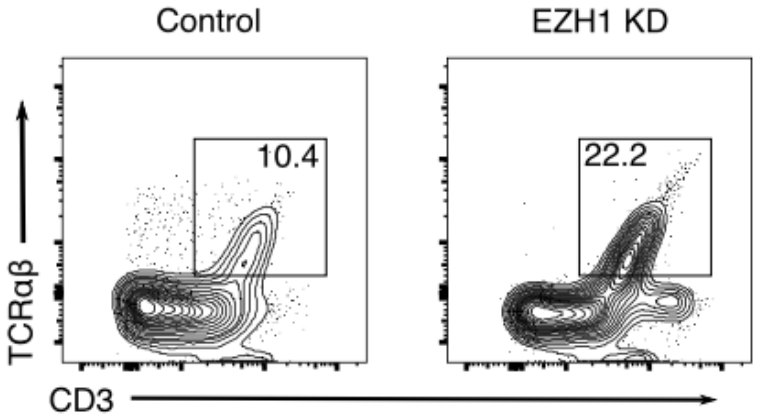


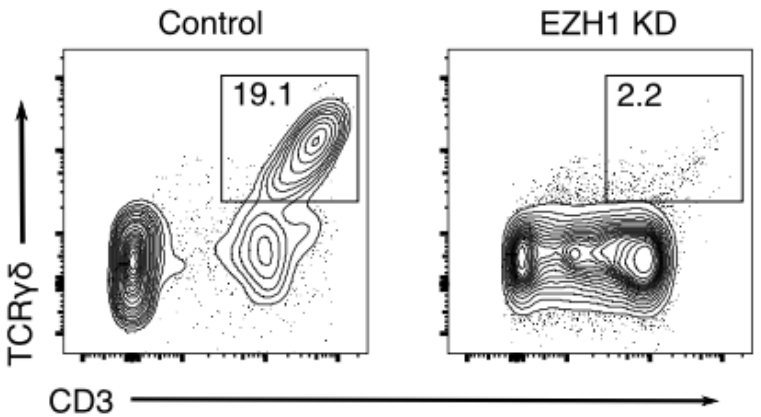
Stroma-free T cell differentiation from iPSCs

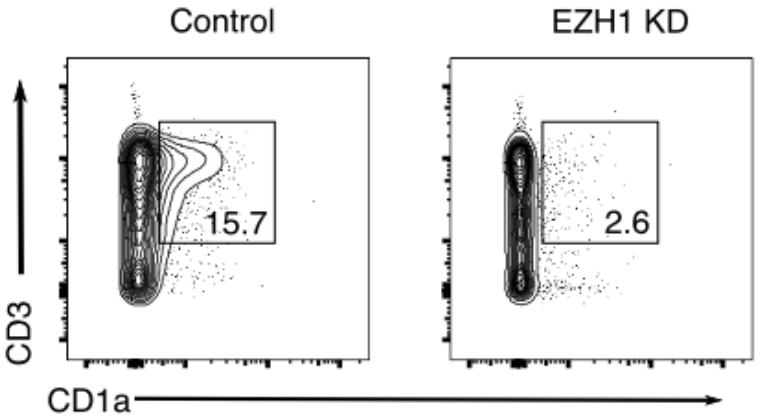


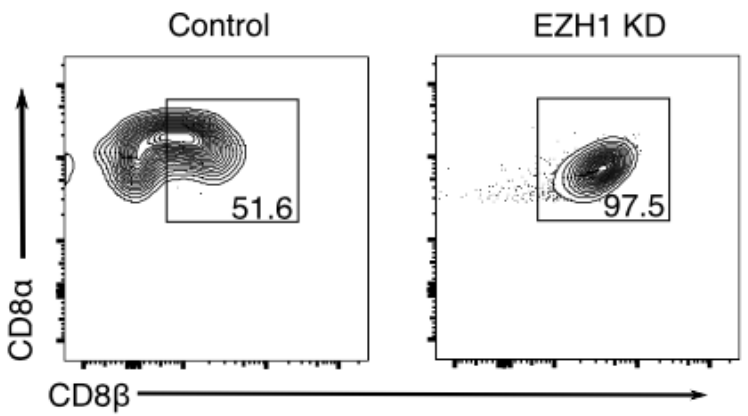
iPSC-derived T cells exhibit mature T cell phenotypes



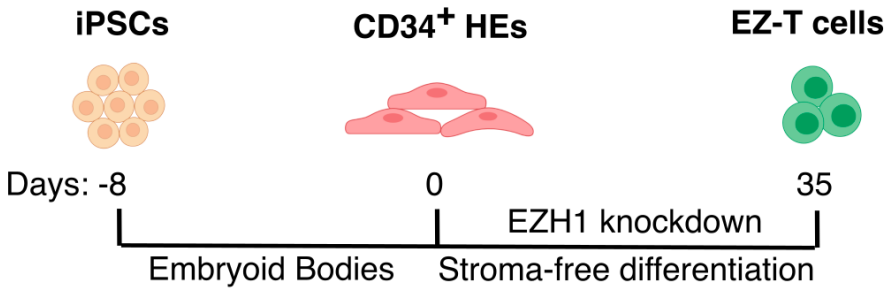




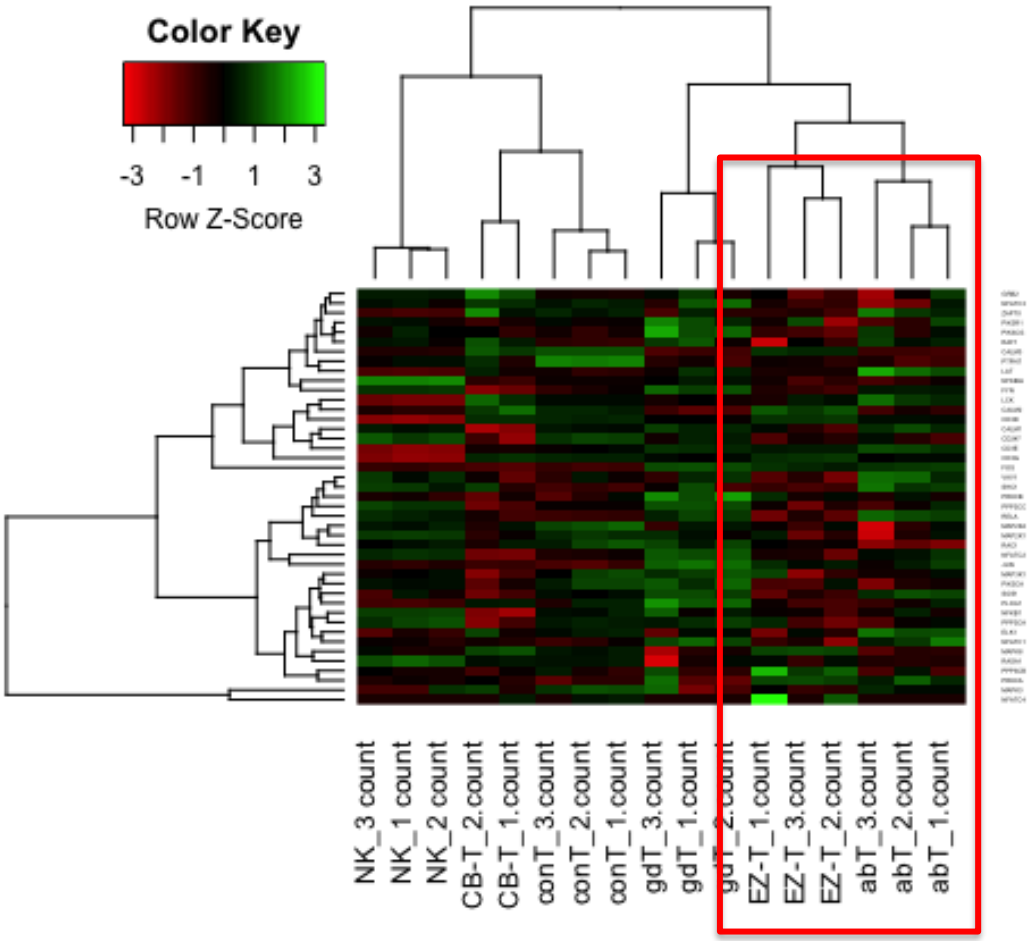




EZ-T cells exhibit molecular signatures of PBMC αβ T cells

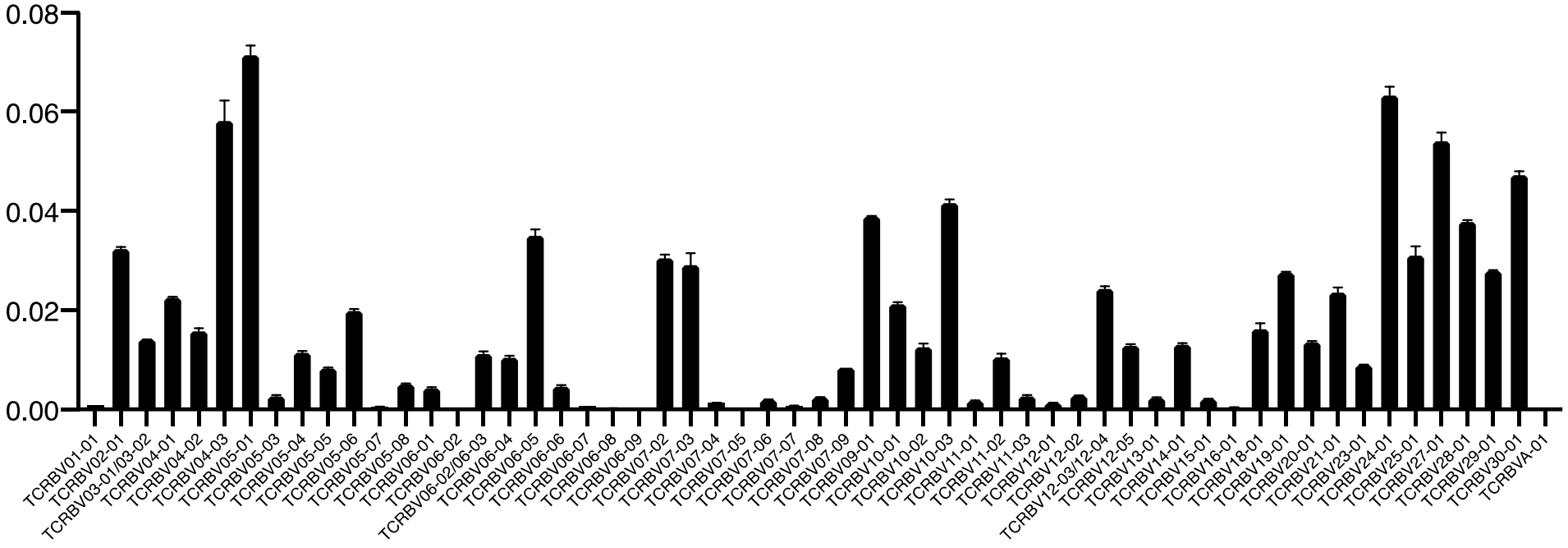


PBMCs → αβ T cells
PBMCs → γδ T cells
PBMCs → NK cells
Cord blood HSPCs → CB_T cells
iPS-HSPCs → Con_T cells
iPS-HSPCs → EZ_T cells



EZ-T cells display a highly diverse TCR repertoire

TCRBV gene usage:



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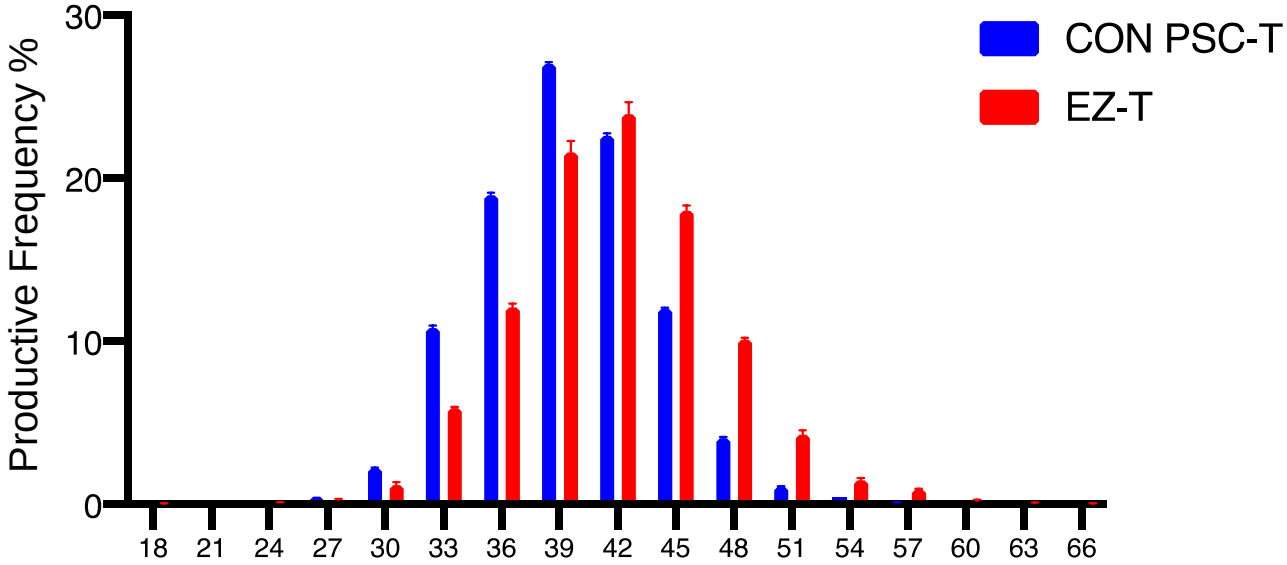
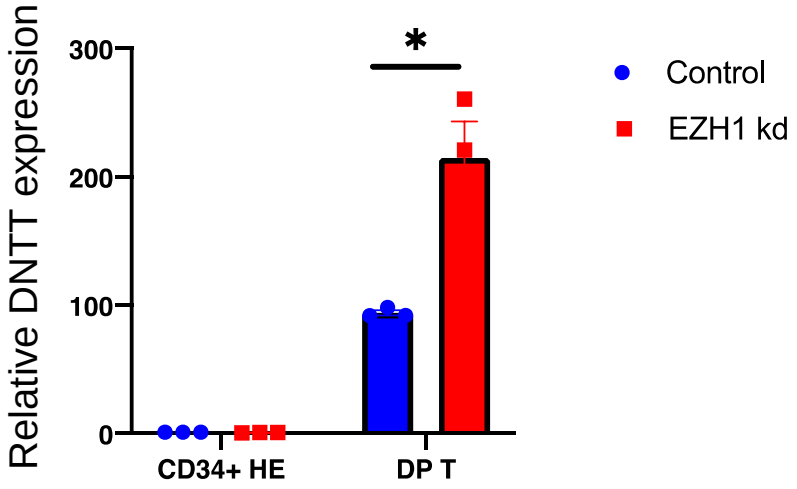
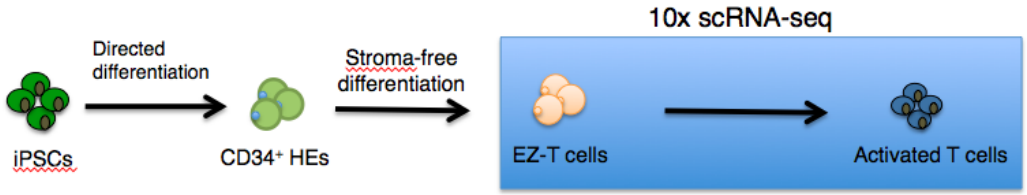
CDR3 length:





EZ-T cells give rise to effector and memory-like subsets upon activation



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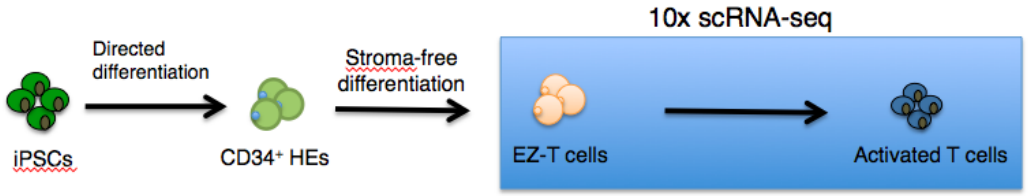

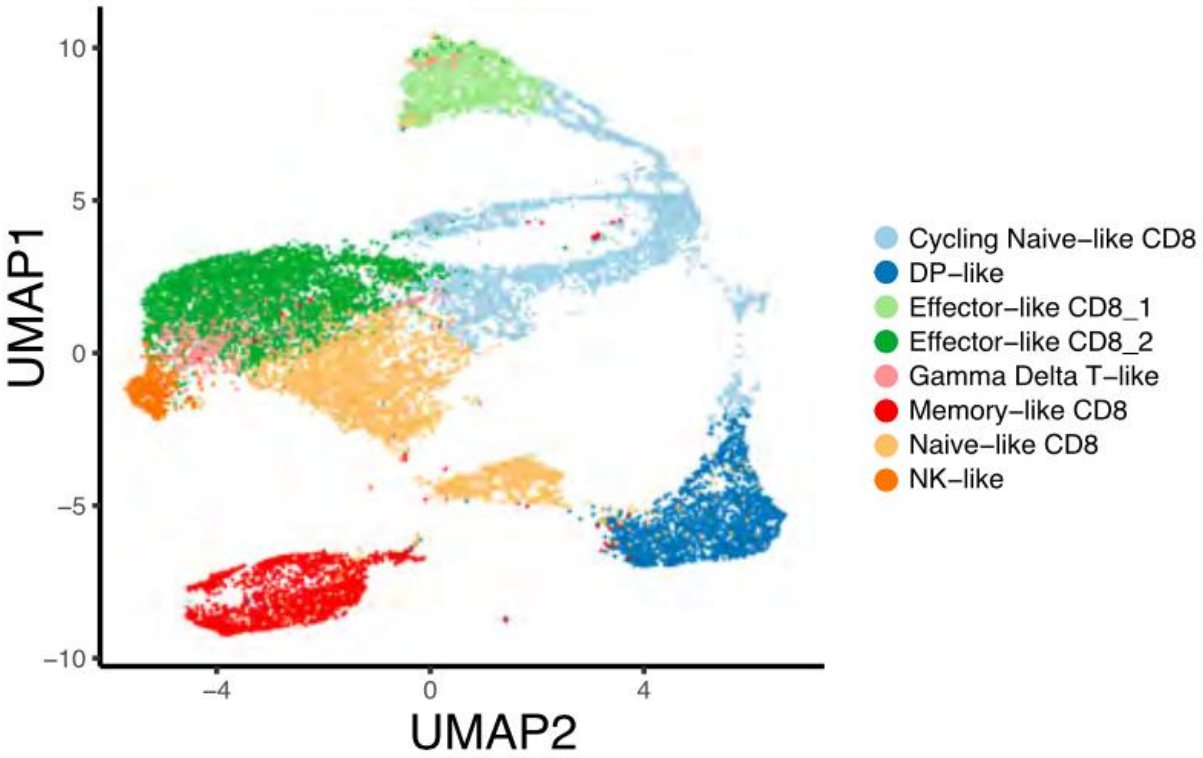

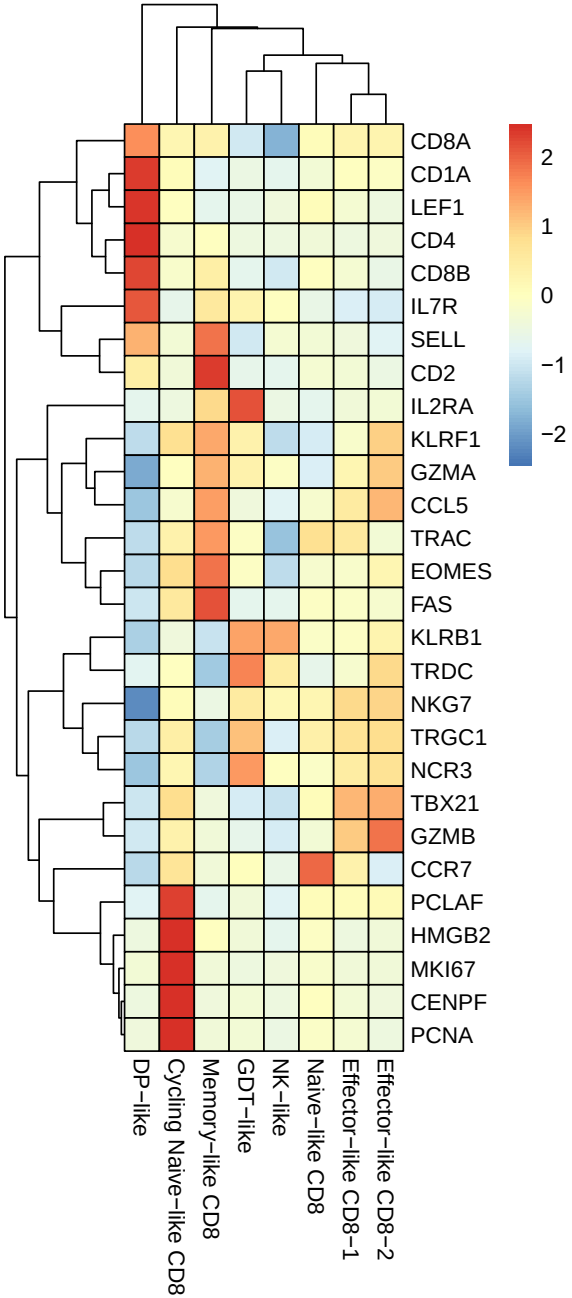

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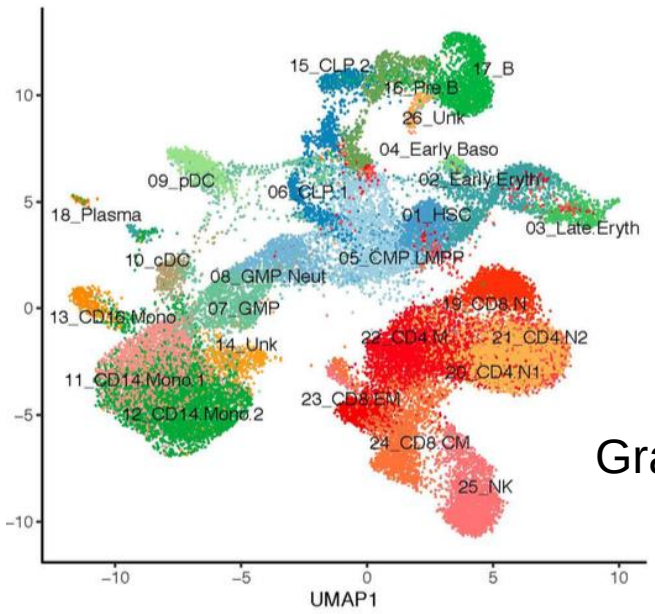
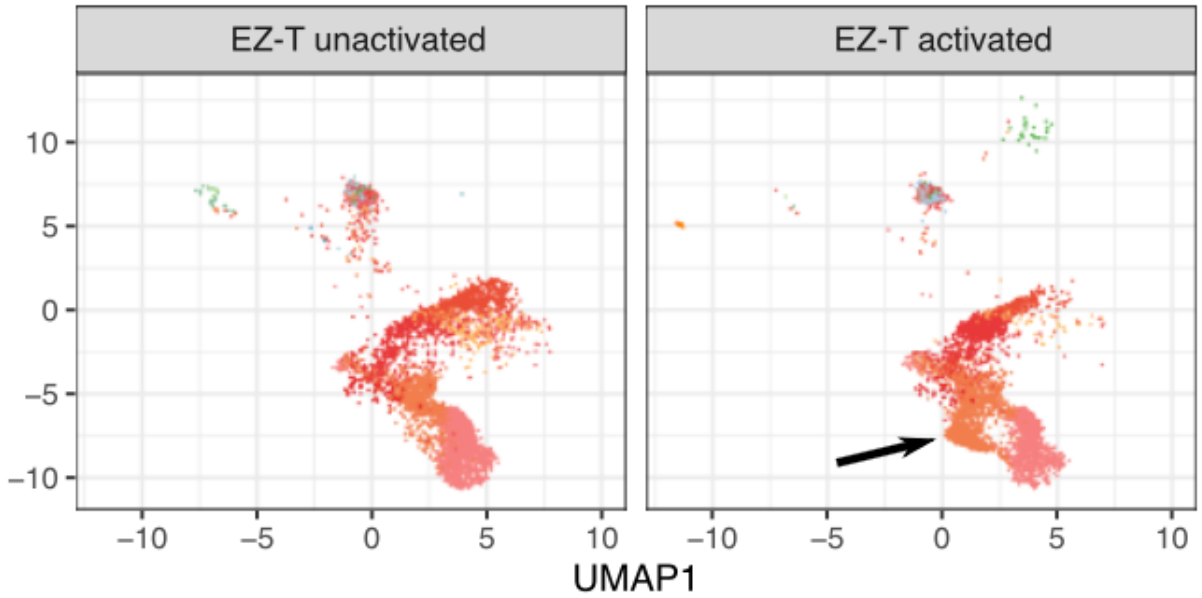
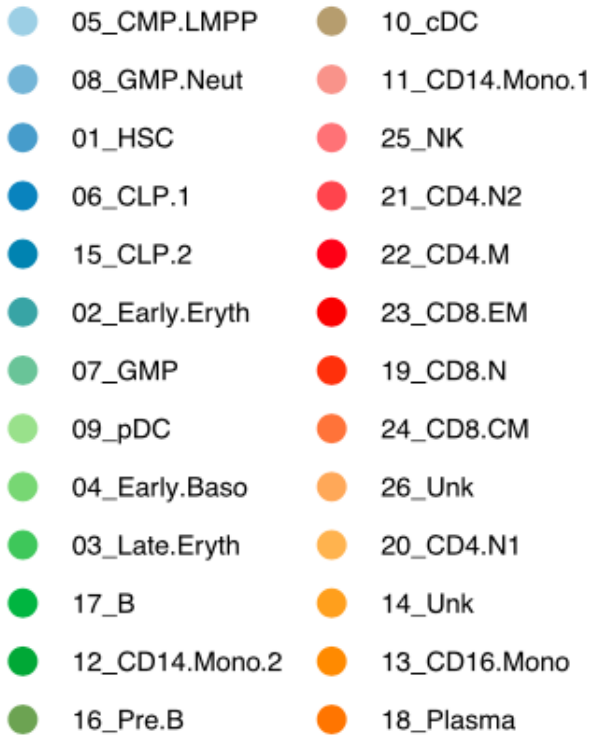
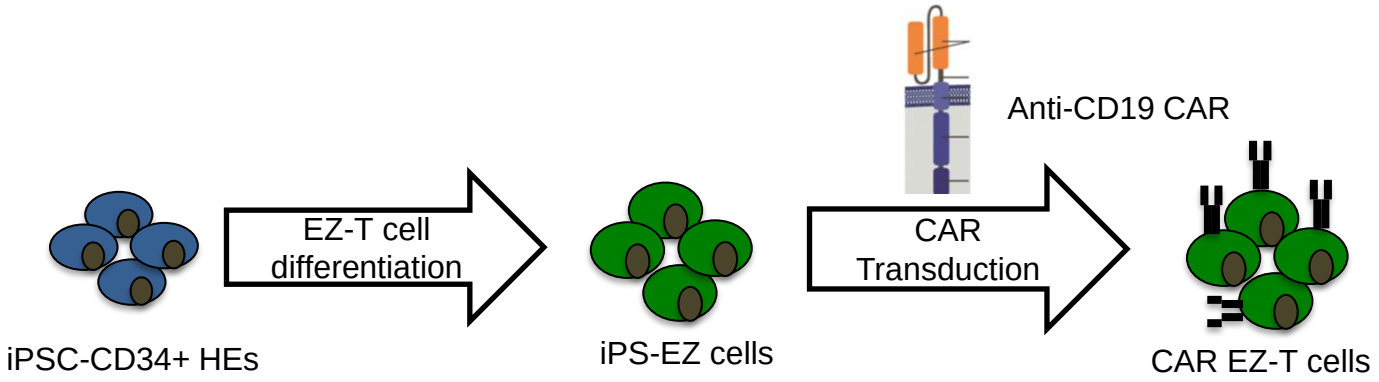


Granja et al., 2019





CAR-transduced EZ-T cells display enhanced effector functions than control iPSC-T cells



CD19-41BBz

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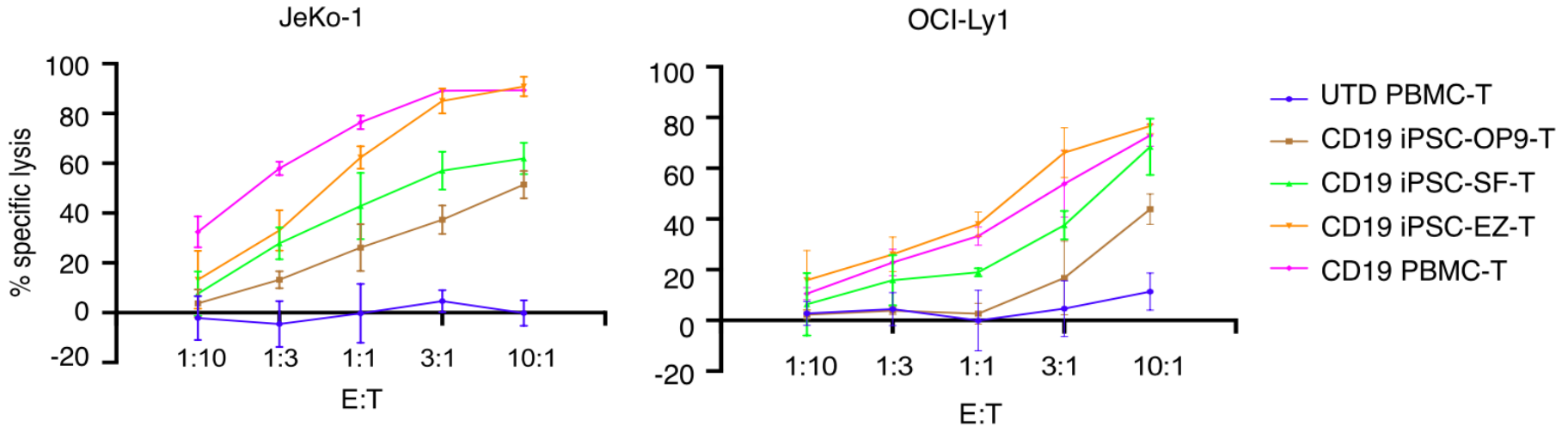
Cytotoxic assay:



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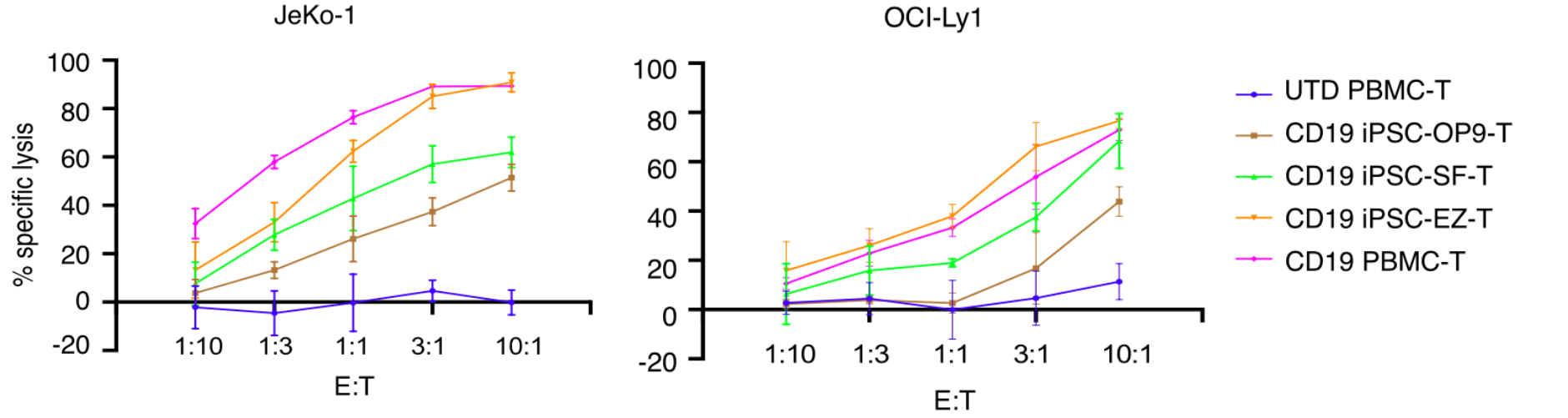
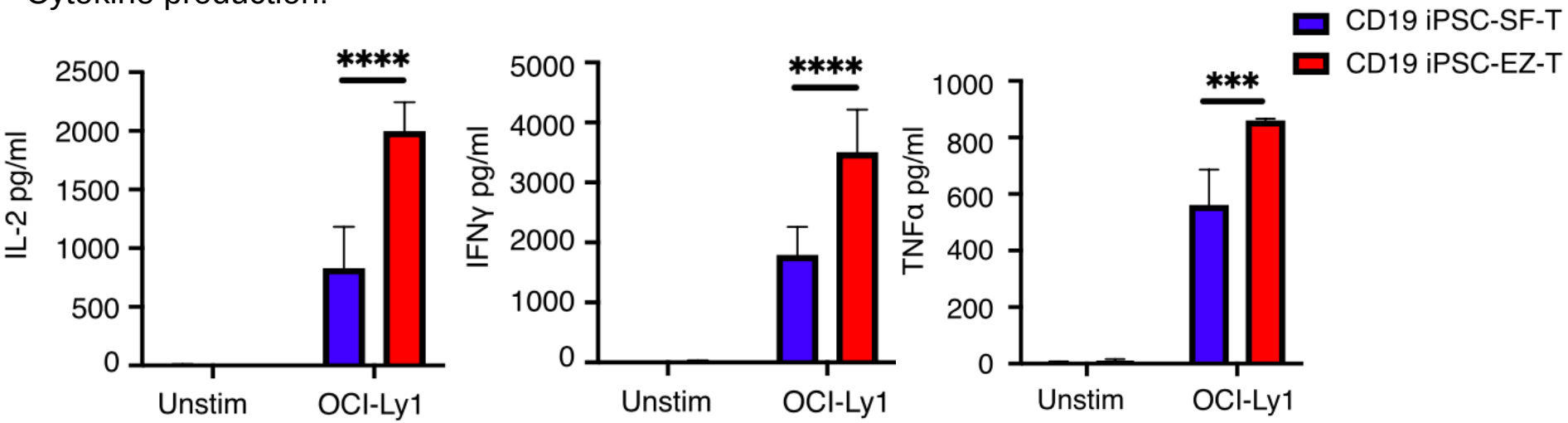
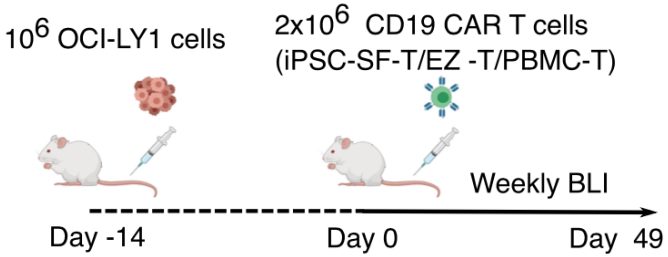


Cytokine production:



CD19 CAR EZ-T cells display superior anti-tumor activity *in vivo*



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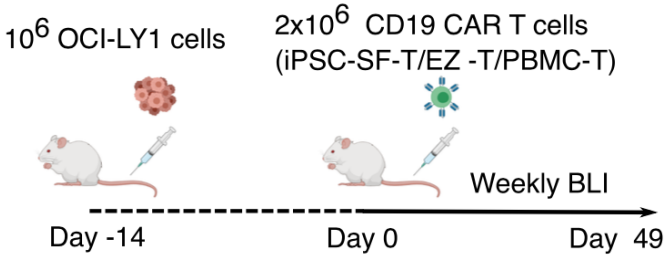

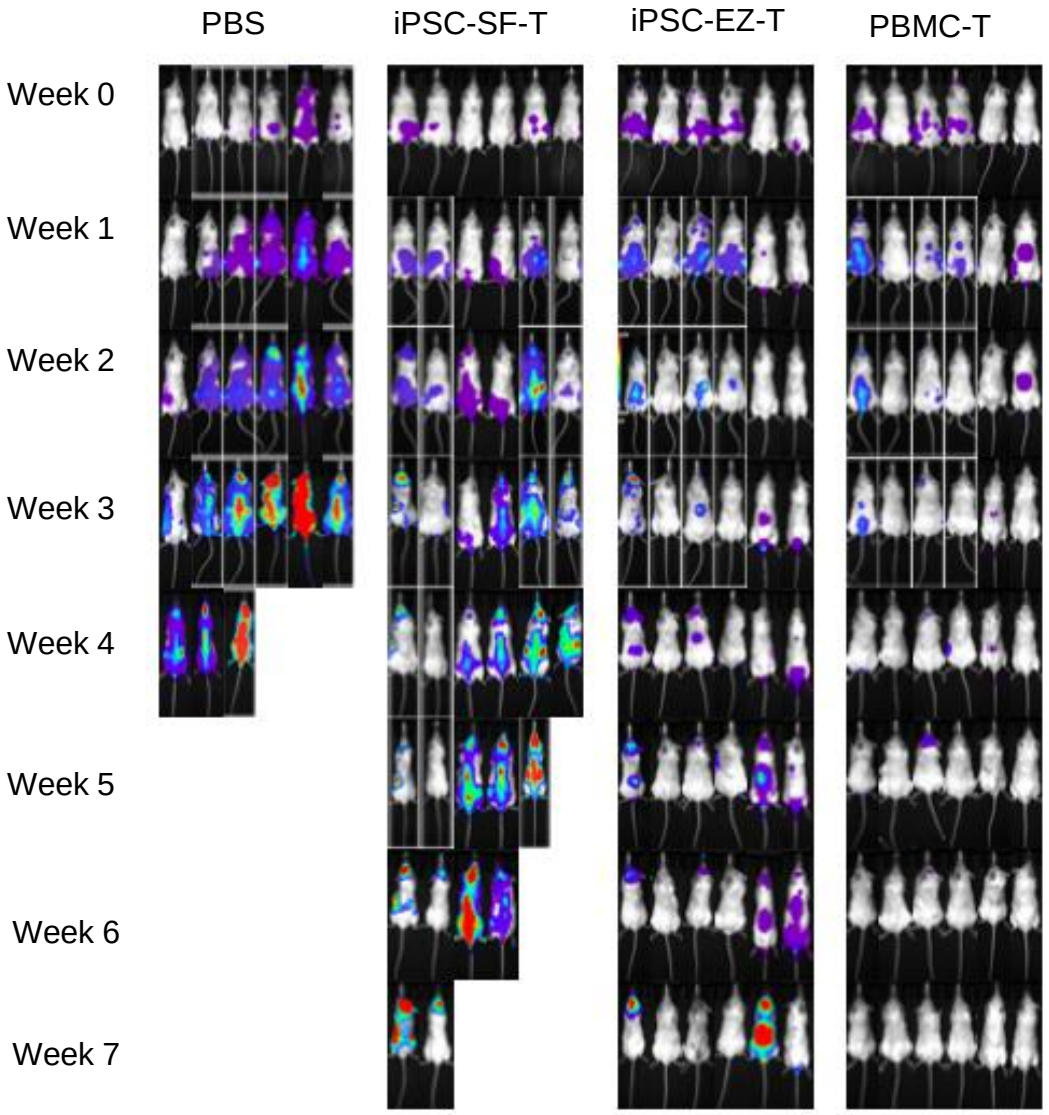

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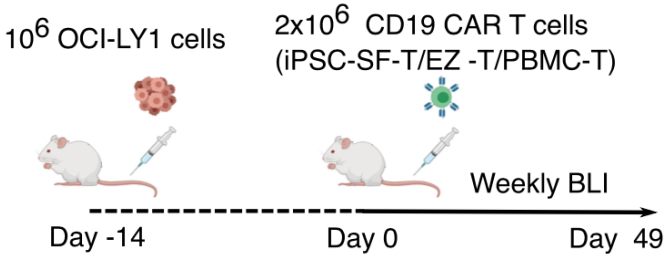
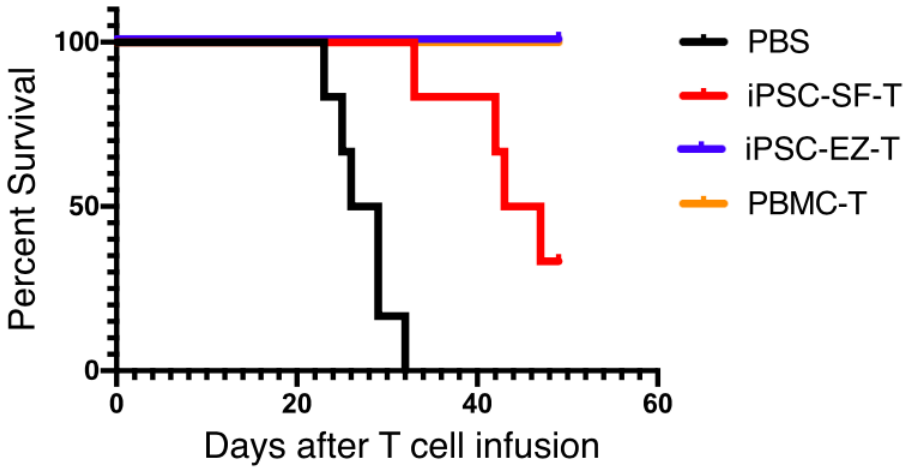
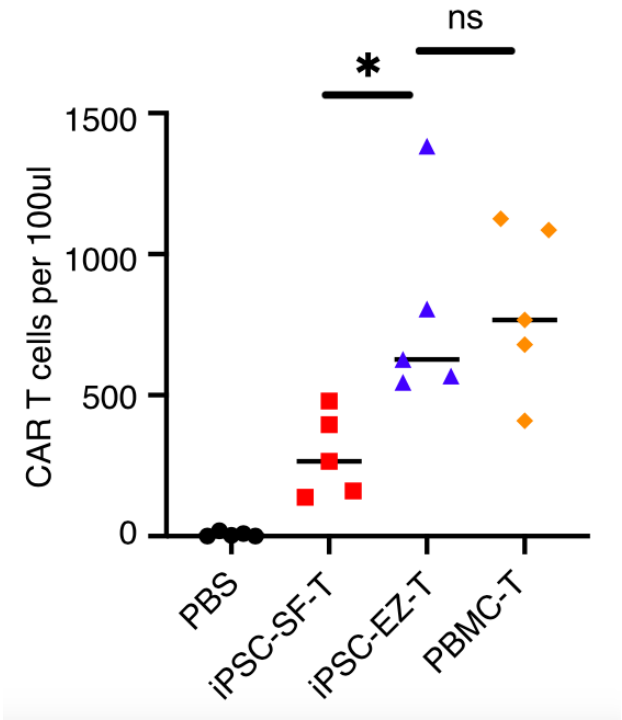
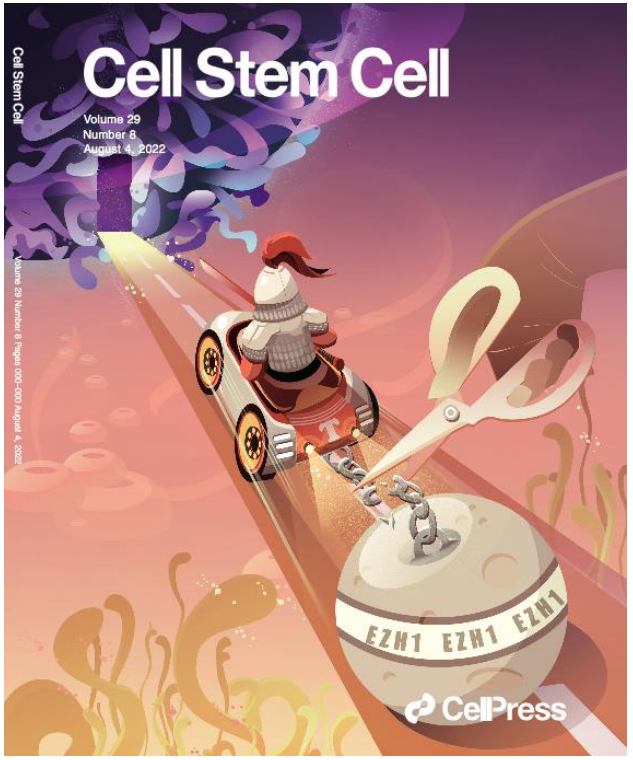






SUMMARY:

- A stroma-free system for efficient production of mature iPSC-T cells expressing diverse TCRs
- EZH1 repression-mediated epigenetic reprogramming generates mature EZ-T cells similar to peripheral blood TCRαβ T cells
- EZ-T cells can give rise to memory-like T cells upon activation.
- CAR EZ-T cells display enhanced antitumor activity in vitro and in vivo



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