950 EPIGENETIC REPROGRAMMING OF IPSC-DERIVED T CELLS FOR CAR T CELL THERAPY

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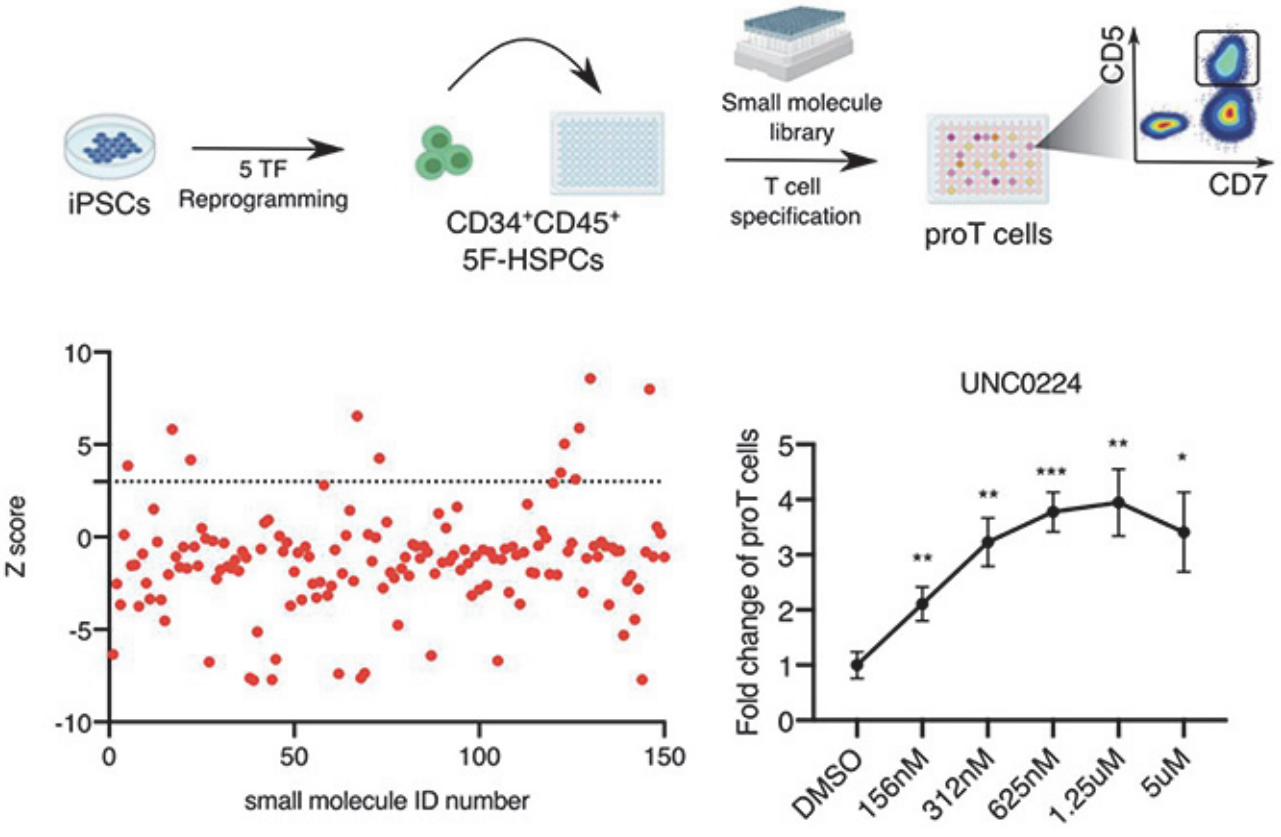
Background Cancer immunotherapy using T cells engineered with chimeric antigen receptors (CAR) has proven remarkably effective against lymphoid malignancies. However, broader application has been impeded by the cumbersome, labor-intensive protocols for engineering autologous patient-specific cells. Human induced pluripotent stem cells (iPSCs) represent an appealing source for scalable manufacture for cell therapy, but deriving mature T cells from iPSCs remains challenging, as iPSC-T cells display features of innate-like gamma-delta T cells and are not as robustly functional as peripheral blood-derived alpha-beta T cells.

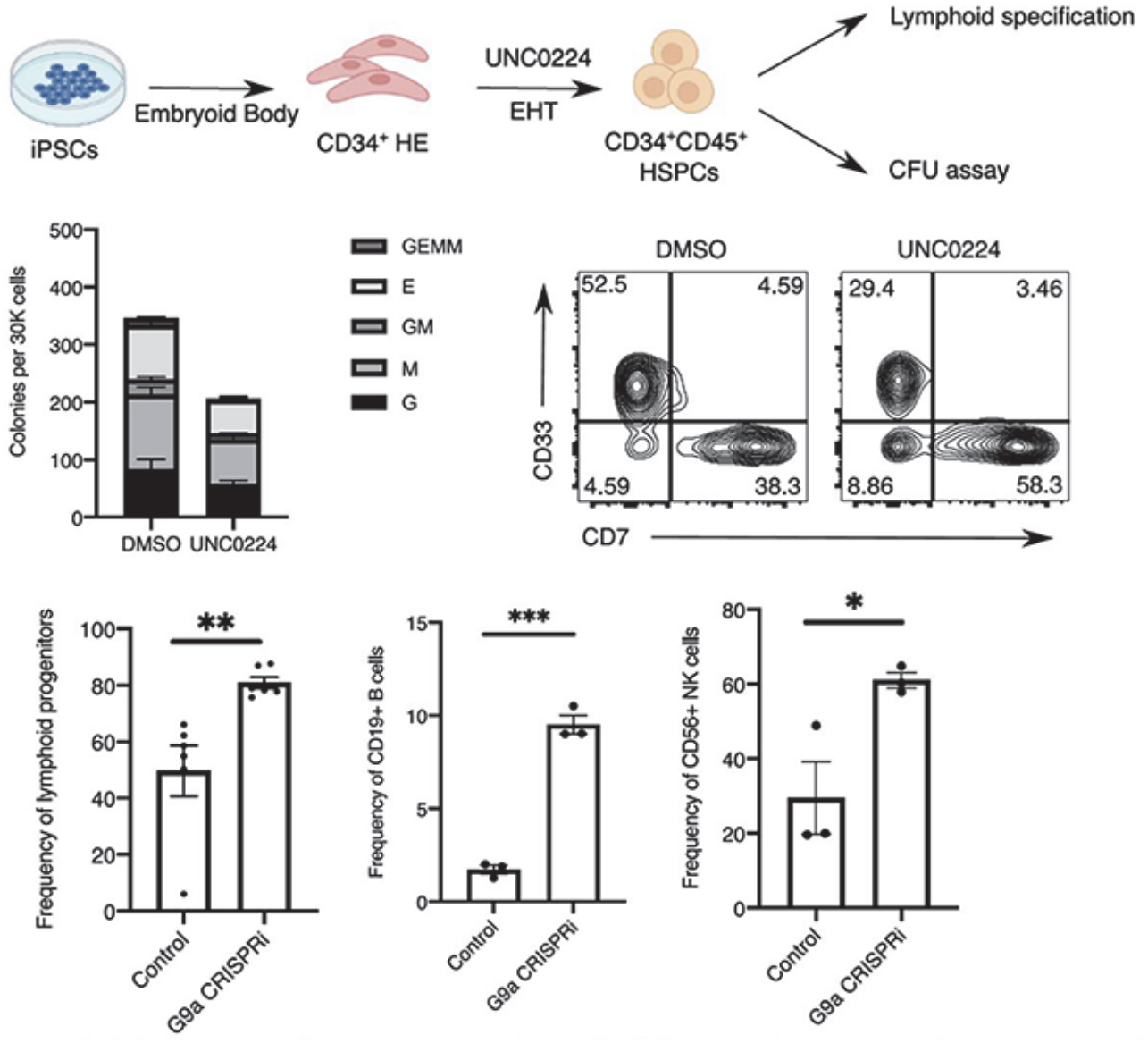
Methods Our lab has established a stroma-free differentiation system that faithfully recapitulates T cell development in culture. Here we performed small molecule screens during iPSC-T cell differentiation to discover new epigenetic regulators that can affect lymphoid development. We then mechanistically examined how epigenetic regulations govern lymphocyte fate decisions via modulating chromatin structure. Finally, we used small molecule-mediate epigenetic modulation to facilitate the production of mature iPSC-T cells with enhanced function.

Results By screening a library of small molecules with known modes of action during iPSC-T cell differentiation, we found that inhibition of histone lysine methyltransferase G9a promotes the production of T cells from iPSC-derived hematopoietic stem and progenitor cells (figure 1). Moreover, we investigated the impact of G9a inhibition on T cell differentiation and lymphoid development in both human iPSCs (figure 2) and zebrafish (figure 3) models, and demonstrated that G9a acts as a repressor of lymphoid potential during the cell fate commitment. ATAC-seq analysis further shows that G9a inhibition regulates chromatin accessible regions associated with T cell differentiation and function (figure 4). By incorporating the small molecule-mediated G9a repression into the T cell differentiation protocol, we generated iPSC-T cells that display a molecular signature similar to mature peripheral blood T cells. Moreover, iPSC-T cells derived via G9a repression exhibit more robust effector response and antitumor activity when engineered with anti-CD19 CAR (figure 5).

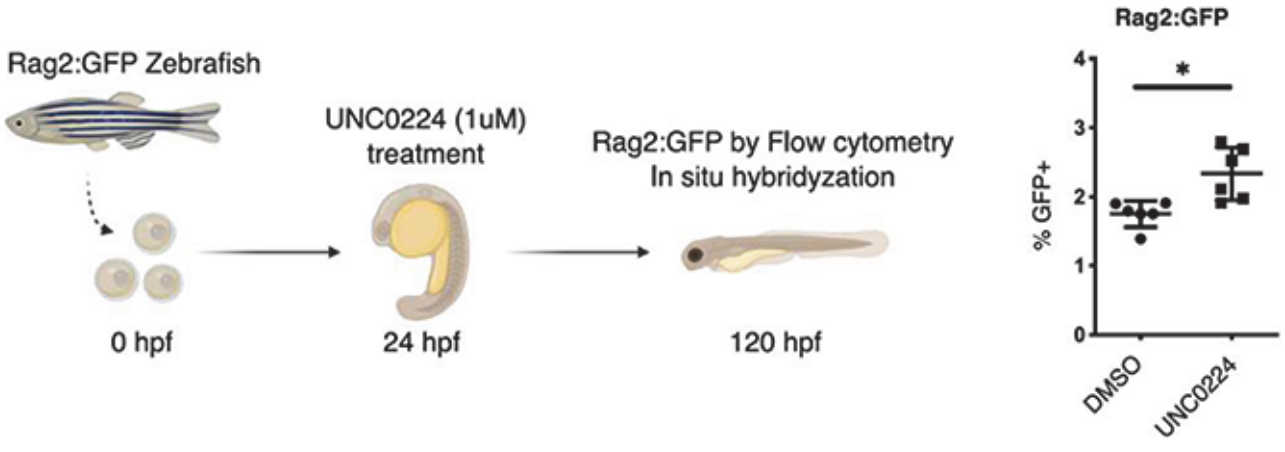
Conclusions Combining in vitro iPSC differentiation with unbiased small molecule screening leads to the discovery of novel mechanisms by which epigenetic regulators affect lymphoid development. Leveraging these new insights, we use small molecule epigenetic modulators to facilitate the generation of mature, functional iPSC-T cells that could be used to realize 'off-the-shelf' stem cell-based CAR T cell therapy.



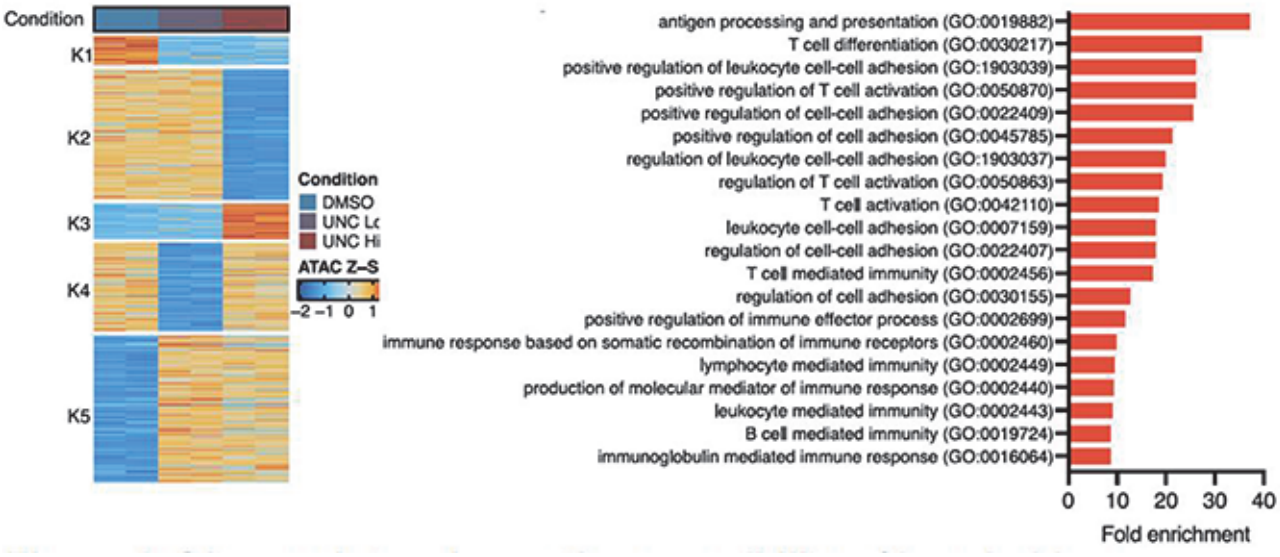
Abstract 950 Figure 1 Small molecule screens identify epigenetic modulators that promote in vitro T cell specification



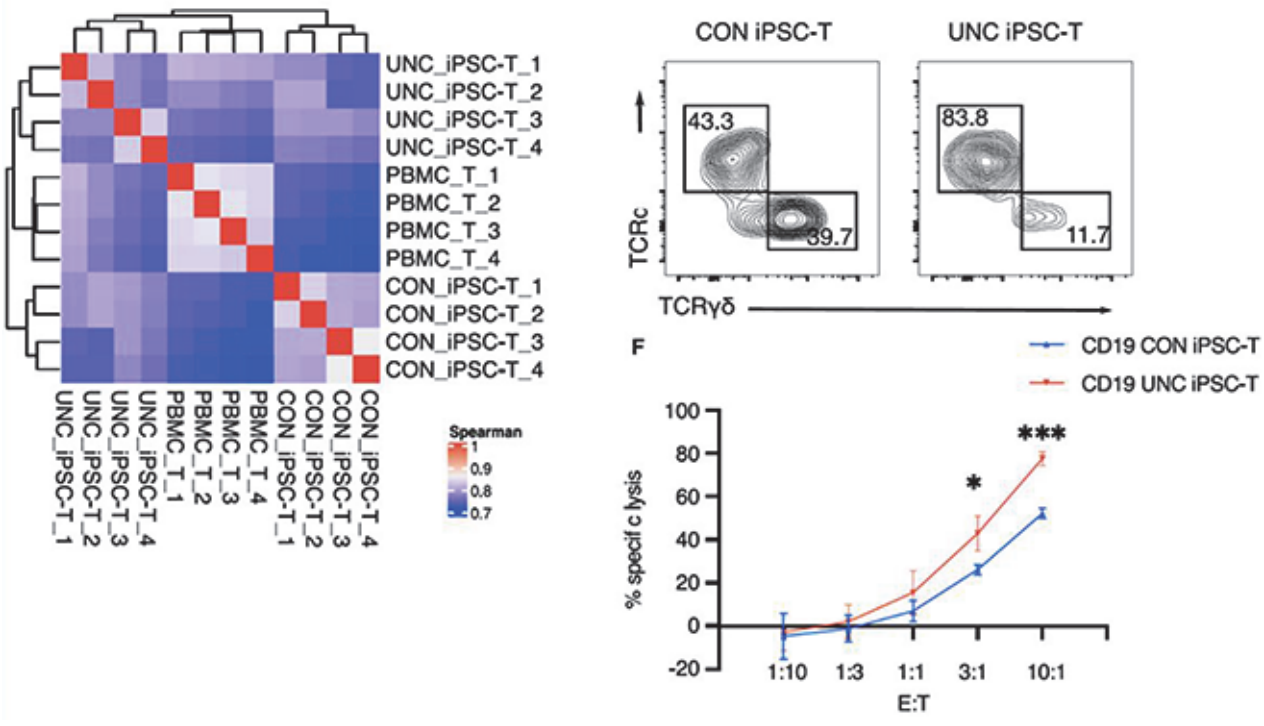
Abstract 950 Figure 2 G9a repression promotes lymphoid commitment at the expense of myeloid potential



Abstract 950 Figure 3 G9a inhibition promotes lymphoid development during zebrafish embryonic hematopoiesis



Abstract 950 Figure 4 G9a regulates chromatin accessibiity of lymphoid genes



Abstract 950 Figure 5 G9a inhibitor during T cell specification facilitates production of mature, functional iPSC-T cells

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