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TETHERED IL-15 TO AUGMENT THE THERAPEUTIC POTENTIAL OF T CELLS EXPRESSING CHIMERIC ANTIGEN RECEPTOR: MAINTAINING MEMORY POTENTIAL, PERSISTENCE, AND ANTITUMOR ACTIVITY

Lenka Hurton

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by

Lenka Victoria Hurton, M.S.

APPROVED:

Laurence J.N. Cooper, M.D., Ph.D.,
Supervisory Professor

Joya Chandra, Ph.D.

Gianpietro Dotti, Ph.D.

Dean A. Lee, M.D., Ph.D.

Bradley McIntyre, Ph.D.

Cliona Rooney, Ph.D.

APPROVED:

Dean, The University of Texas
Graduate School of Biomedical Sciences at Houston

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A

DISSERTATION

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Lenka Victoria Hurton, M.S.

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DEDICATION

To my husband and best friend, Vin,

for believing in me more than I believed in myself,

for all of his patience,

and for selflessly supporting the pursuit of my career.

I couldn’t have done this without you.

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ABSTRACT

Tethered IL-15 to augment the therapeutic potential of T cells expressing chimeric antigen receptor: Maintaining memory potential, persistence, and antitumor activity

Adoptive immunotherapy can retarget T cells to CD19, a tumor-associated antigen (TAA) expressed on B-cell malignancies, by the expression of a chimeric antigen receptor (CAR). Infusion of CAR-modified T cells for the treatment B-cell malignancies has demonstrated promise in preclinical and clinical trials. These data highlight the ability of infused CD19-specific T cells to be synchronously activated by large burdens of $CD19^+$ leukemia and lymphoma. This can lead to dramatic antitumor effects, but also exposes the recipient to toxicity associated with tumor-cell lysis and cytokine storm. Clinical trials will now be addressing the targeting of minimal burdens of $CD19^+$ malignancy as patients are enrolled earlier in their disease course and receive concomitant chemotherapy. It is likely that the existing populations of CAR T cells generated *ex vivo* to address relapsed disease may not be able to address minimal residual disease (MRD). Therefore, we have developed a clinically appealing approach to sustaining the persistence of CAR T cells independent of TAA by providing signaling through the common gamma chain receptor (γc). Administration of exogenous soluble recombinant cytokines that signal through the γc, such as interleukin (IL)-2, have been used clinically to sustain the persistence of adoptively transferred T cells. However, systemic high-dose administration has resulted in dose-limiting toxicities. Unlike IL-2, IL-15 possesses numerous attributes desirable for adoptive therapy and has been ranked among the most valuable immunotherapeutic agent for cancer treatment. It is a pro-survival cytokine that promotes the survival of long-lived T-cell memory subsets and *in vivo* antitumor activity. Unlike other γc family cytokines, IL-15 is transpresented to responding T cells in the context of IL-15 receptor alpha (IL-15Rα). Therefore, we *hypothesized* that a membrane-bound IL-15 fusion protein (mIL15) tethered to the cell surface would enhance T-cell costimulation to support persistence independent of CAR activation by preserving T-cell memory potential and maintain antitumor activity in the presence of low TAA. Using clinically compliant methods, the generated mIL15-CAR T cells mimicked the physiologic mechanism of transpresentation to sustain costimulation via phosphorylation of signal transducer and activator of transcription (pSTAT5). In contrast to conventional CD19-specific CAR T cells, mIL15-CAR T cells persisted in mice independent of the presence of TAA and mediated potent rejection of a systemically distributed $CD19^+$ leukemia. The potential for sustained immunity against B-cell malignancies was shown as, in the absence

of antigen, mIL15-CAR T cells were long-lived and adopted a desirable CD45RO^{neg}CCR7^{+} “low-differentiation” state with a memory-like molecular profile and phenotype. These results have direct implications for the design of an adoptive immunotherapy clinical trial evaluating mIL15-CAR T cells in the setting of MRD and warrants further investigation of mIL15 to engineer T cells targeting other tumor cells that have sequestered or low levels of TAA.

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LIST OF ABBREVIATIONS

ACT: Adoptive Cell Therapy

AICD: Activation Induced Cell Death

Akt: Protein Kinase B (PKB)

ALL: Acute Lymphoblastic Leukemia

Allo-HSCT: Allogeneic-hematopoietic Stem Cell Transplantation

AML: Acute Myeloid Leukemia

aAPC: artificial Antigen Presenting Cell

APC: Antigen Presenting Cell

BCL-6: B Cell Lymphoma 6

BLI: Bioluminescence Imaging

BLIMP-1: B Lymphocyte Maturation-induced Protein 1

CAR: Chimeric Antigen Receptor

CD: Cluster of Differentiation

CLL: Chronic Lymphocytic Leukemia

CML: Chronic Myeloid Leukemia

CRA: Chromium Release Assay

EOMES: Eomesdermin

FBS: Fetal Bovine Serum

ffLuc: *Firefly Luciferase*

FOXO: Forkhead Box O

γc: Common Gamma Chain Receptor (CD132)

GSK-3β: Glycogen Synthase Kinase 3β

GVHD: Graft-Versus-Host-Disease

GVL: Graft –Versus-Leukemia

HSCT: Hematopoietic Stem Cell Transplantation

IFNγ: Interferon Gamma

JAK: Janus Family Tyrosine Kinase

IL: Interleukin

LAC: Lymphocyte Activating Cocktail

MFI: Mean Fluorescence Intensity

mIL15: Membrane-bound IL-15 and IL-15Rα Fusion Protein

MRD: Minimal Residual Disease

mTORC1: Mammalian Target of Rapamycin Complex 1

NF-κB: Nuclear factor-κB

NK cell: Natural Killer cell

NSG: NOD.*scid.*$\gamma_c^{-/-}$ Genotype Mice

PBMC: Peripheral Blood Mononuclear Cell

pERK: Phosphorylated Extracellular-signal-regulated Kinase

pSTAT: Phosphorylated Signal Transducer and Activator of Transcription

PCR: Polymerase Chain Reaction

PI3K: Phosphoinositide 3-kinase

pSBSO: *Sleeping Beauty* transposon plasmid

rLuc: *Renilla Luciferase*

RPMI: Roswell Park Memorial Institute Medium

SB: *Sleeping Beauty*

scFv: Single-chain Variable Fragment

STAT: Signal Transducer and Activator of Transcription

Stim: T Cells Stimulated/Co-cultured with aAPC

T_{CM}: Central Memory T Cell

T_{EFF}: Effector T Cell

T_{EM}: Effector Memory T Cell

T_{EMRA}: Effector Memory RA T cell

T_N: Naïve T Cell

T_{REG}: Regulatory T Cell

T_{SCM}: Memory Stem Cell

TAA: Tumor-associated Antigen

TCR: T-cell Receptor

TGF-β: Transforming Growth Factor-β

WD-T Cell: CAR-modified T cells that have undergone 65 days of antigen withdrawal

CHAPTER 1

Background

1.1 B-cell Acute Lymphoblastic Leukemia

B-lineage acute lymphoblastic leukemia (ALL) occurs in both pediatric and adult populations where approximately 60% of B-ALL cases are in individuals younger than 20 years old (1, 2). This tumor cell type typically initiates at the pro-B cell stage where development is halted and the cells retain progenitor characteristics without further differentiation to a mature state (3). Initial induction therapy with chemotherapeutics is aggressive, toxic, and long-term (4). Chemotherapeutic treatment of adult and pediatric ALL have disease relapse rates of 65% and 20%, respectively, due to drug-resistant residual disease (5, 6). Adults have poor prognosis, where greater than 60% will succumb to their disease, but about 80% of pediatric patients have long-term survival (7). However, children who relapse have a cure rate of 25-40% (8). As leukemia is the most frequently occurring childhood malignancy at 34% (9), though, the absolute number of relapses is substantial and makes it one of the most common pediatric malignancies (10, 11). Especially concerning for pediatric patients are the late treatment effects that may have to be fought years after treatment. The aggressive induction treatments, and even some of the less toxic therapies introduced more recently, increase the risks of other chronic and serious health conditions of childhood cancer survivors and can include organ impairment and secondary cancers (12). The high incidence of ALL relapse, especially in poor prognostic groups such as those with very early relapse or $CR3^{+}$ (13, 14), coupled with the associated long-term health effects of aggressive chemotherapeutic treatment has prompted the use of immune-based therapies.

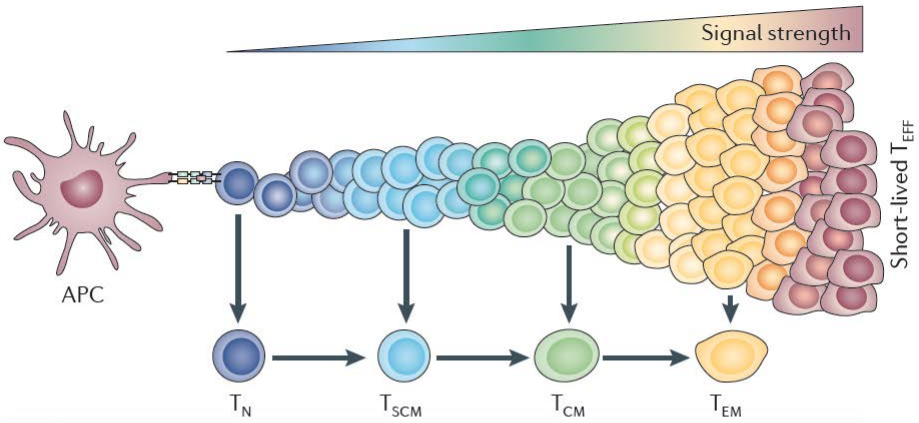
1.2 CD8 T Cell Differentiation During Immune Response

Cytotoxic T lymphocytes (CTLs) are a component of the adaptive immune system that participates in the elimination of cells that have been infected by virus or intracellular bacteria. Quiescent naïve T cells residing in secondary lymphoid organs can be described as $CD45RA^{+}CCR7^{+}CD62L^{-}CD27^{+}CD28^{+}$ (15). Upon being alerted to pathogen, antigen presenting cells (APC) interact with naïve CD8 T cells in secondary lymphoid organs and

initiate the expansion phase of antigen-specific T cells that will go on to address clearance of the viral or intracellular bacteria (16). In a progressive T cell differentiation model, priming of the naïve T cells (T_N) results in activation, proliferation, and phenotypic changes ultimately endowing them with effector function (**Fig. 1**) (17). CD45RO expression when paired with CD62L and CCR7 signifies central memory T cells (T_{CM}), which traffic amongst lymphoid organs and have the hallmark capability of producing IL-2 (18, 19). As, differentiation continues, CD62L and CCR7 are lost, signifying effector memory cells (T_{EM}), which are relegated to trafficking in peripheral tissues and are capable of producing copious levels of IFNγ (18, 20). The further along the differentiation path the cell is, the greater the acquisition of effector function, but it is concomitant with a loss of longevity. Progression through the stages of differentiation is characterized by stepwise changes in phenotype and loss of function that ultimately impacts efficacy in clearing pathogen or, as in the topic of this dissertation, clearance of tumor (21, 22). The contraction phase follows pathogen clearance where CD8 T cells quickly become apoptotic and die, leaving only 5-10% to remain in the memory T cell pool (16).

The road travelled between the initial naïve state of a T cell and the final terminally differentiated T cell that dies, is under considerable debate. Several models have been proposed to explain T cell differentiation and diversification: separate-precursor model, decreasing–potential model, signal–strength model, asymmetric cell fate model (reviewed in (16, 23)). They aim to explain how heterogeneous populations of short-lived effector and long-lived memory CD8 T cells are formed during an immune response, but no consensus has been reached in making a model determination since numerous experiments support each of the models. Moreover, these conflicting studies have been done in models of viral or bacterial infection and thus studying the determination of T cell lineage in tumor models may contribute to this debate and it has remained relatively unexplored.

What there is more agreement about is that intrinsic factors are modulated throughout T cell progression from naive to long-lived memory precursors to short-lived terminally differentiated effector cells (reviewed in (16, 17, 24). Alteration of transcription factor levels is not necessarily an on-off switch, but can take the form of a reciprocal gradient of transcriptional programs controlling effector and memory differentiation. Well-known axes are: eomesdermin (EOMES) opposing T-bet and B cell lymphoma 6 (BCL-6) opposing B lymphocyte maturation-induced protein 1 (BLIMP-1), where EOMES and BCL-6 institute memory programs (16). The frequency and intensity of T cell receptor (TCR) induction from antigen encounter, signals from costimulatory molecules such as



Phenotype	CCR7	+++	+++	++	–
	CD62L	+++	+++	++	–
	CD27	+++	+++	++	+/–
	CD28	++	+++	+++	+/–
	CD45RA	+	+	–	+/–
	CD45RO	–	–	+	+
	CD122	–	++	+++	+++
	CD95	–	++	+++	+++

Figure 1. Progressive T cell differentiation model. Naive T (T_N) cells are primed by antigen-presenting cells (APCs) at the initiation of an immune response. The strength and quality of stimulatory signals influences T cell progression along a differentiation pathway that ends in the generation of terminally differentiated short-lived effector T (T_{EFF}) cells. Upon pathogen clearance, antigenic and inflammatory stimuli diminish enabling primed T cells to become quiescent and enter into various memory subsets depending on the intensity of extrinsic signals received. T cell differentiation increasingly progresses from memory stem cell (T_{SCM}), central memory (T_{CM}) cell, to effector memory (T_{EM}). The phenotypic characteristics are illustrated as: not expressed (–), low expression (+), intermediate expression (++), and high expression (+++).

Adapted from: Gattinoni L, Klebanoff C, Restifo NP. 2012. Paths to stemness: building the ultimate antitumour T cell. *Nature Reviews Cancer* 12(10). Adapted by permission from Macmillan Publishers Ltd.

CD28, and inflammatory cytokines are extrinsic factors that merge with intrinsic elements to mold T cell differentiation (**Fig. 2**). The phosphatidylinositol 3-kinase (PI3K)/Akt pathway is a central node to which these signals coalesce. Akt integrates these converging signals leading to downstream regulation of the Wnt/β-catenin pathway, forkhead box O (FOXO), glycogen synthase kinase 3 (GSK3), and mammalian target of rapamycin complex 1 (mTORC1) in a rheostat-like manner (25-27). Strong induction of Akt activation institutes a transcription program that drives T cell differentiation and results in enhanced effector function with concomitant decline in differentiation to a memory state and loss of survival potential (26). Gaining further understanding of the modulation of external conditions and manipulation of intrinsic elements is ultimately the key to modulating T cell responses in adoptive cell therapy (ACT). Work in this dissertation aims to evaluate the intrinsic state of genetically-modified T cells.

1.3 Harnessing the Immune System to Target ALL Minimal Residual Disease

CD8 T cells, and other elements of the immune system, are not limited to responding to viral or intracellular bacterial pathogens, but they have also emerged as crucial players in holding back cancer development. This concept has come to be known as cancer immunosurveillance. The immune system can recognize cells that have undergone changes predisposing them to cancer, or that have transformed to malignancy, and eliminate these abnormal cells (28, 29). This process may fail and cancer may then arise but, with intervention and appropriate assistance, the immune system can be reenergized to enable it to return to what it was specifically made to do.

The high incidence of ALL relapse, especially in poor prognostic groups, has prompted the use of immune-based therapies using allogeneic-hematopoietic stem cell transplantation (Allo-HSCT) (30). This therapy is dependent on alloreactive cells present in the donor graft for the eradication of remaining leukemic cells to achieve a graft-versus-leukemia (GVL)-effect to improve disease-free survival (31). Donor lymphocyte infusions have been used to enhance the ability of engrafted T cells to target residual ALL after allo-HSCT, but this treatment approach for patients with ALL achieves less than a 10% remission rate and is associated with a high degree of morbidity and mortality from the frequency and severity of graft-versus-host disease (32, 33). High risk of relapse for

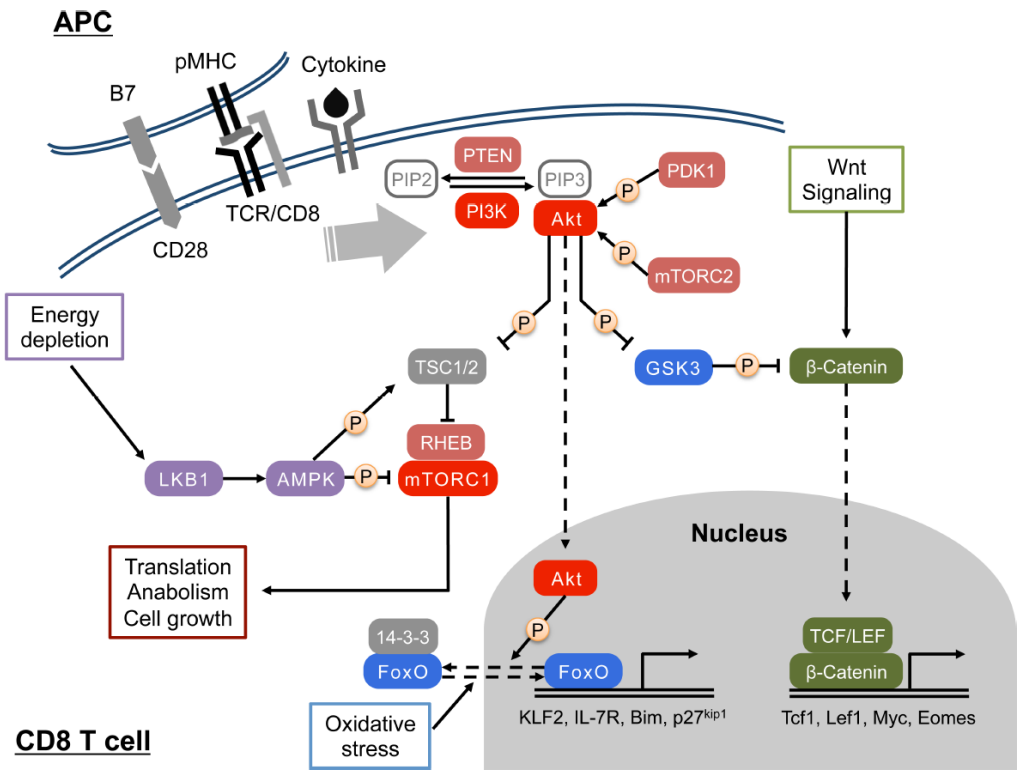


Figure 2. Activation of PI3K/Akt signaling in the CD8 T cell. In CD8 T cells, PI3K activation is initiated by engagement of the TCR with peptide/MHC I complex, signals from costimulation, cytokines, and/or chemokines. Functioning at the plasma membrane, activated PI3K phosphorylates phosphatidylinositol-4,5-bisphosphate (PIP2) yielding phosphatidylinositol-3,4,5-triphosphate (PIP3), which recruits PH domain-containing proteins such as Akt. Phosphoinositide-dependent protein kinase (PDK1) and mammalian target of rapamycin complex 2 (mTORC2) are required for full activation (phosphorylation) of the Ser/Thr kinase Akt. Upon activation, Akt localizes to and functions in the cytosol. It phosphorylates and inhibits tuberous sclerosis complex 1/2 (TSC1/2), which is part of a negative regulatory complex of mTORC1. Resultant mTORC1 activity initiates protein synthesis and cell growth via S6K and 4E-BP. Akt also inhibits GSK3 which regulates the canonical Wnt/β-Catenin pathway. Additionally, Akt translocates to the nucleus to phosphorylate FOXO transcription factors resulting in their nuclear export that plays a role in cell quiescence, apoptosis, and memory.

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particular malignancies has been observed in pediatric transplants with high risk ALL and Ph^+ALL transplants achieving 58% and 37% disease free survival (34, 35). With relapse still a common and lethal problem in these refractory malignancies, adoptive therapy using T cells after HSCT may be used to target residual leukemic cells. Increasing the GVL-effect toward the minimal residual disease (MRD) can be accomplished by redirecting the specificity of donor T cells.

1.4 Adoptive Immunotherapy: Redirecting T Cell Specificity

In the absence of a tumor-associated antigen and/or a T cell expressing an endogenous $\alpha\beta$ TCR with specificity for a tumor, T-cell therapy hinges on the ability to genetically engineer cells with redirected antigen specificity. Manipulating T cell specificity toward a tumor antigen is possible by the introduction and the expression of a chimeric antigen receptor (CAR). Infusion of such antigen redirected T cells is developing into a targeted treatment for the elimination of malignant cells and prevention of disease relapse. Numerous formulations of CARs that are specific for several target antigens have been developed with some of these approaches progressing into phase I clinical trials (36).

A CAR is typically composed of an extracellular antibody-derived single-chain variable domain (scFv) for antigen recognition and is joined by a flexible linker connected to a transmembrane domain and an intracellular signaling domain(s) that includes CD3ζ for T-cell activation (**Fig. 3**) (37, 38). In this way, CAR T-cell recognition of tumor targets occurs independently of the major histocompatibility complex enabling their application to be universal (37). Clinical trials using these 1^{st} generation CARs demonstrated the feasibility of adoptive transfers, however while some patients did show evidence of tumor responses others did not respond with lasting effects (BB-IND11411, LJNC) (39, 40). In the study by Park et al. (40), the persistence of CAR-modified T cells was found to be short and ranged from one to seven days in patients with bulky disease. Numerous studies have now demonstrated that T cell persistence correlates with therapeutic efficacy (41-43) and this dissertation aims to address the issue of poor or inconsistent persistence of CAR-modified T cells.

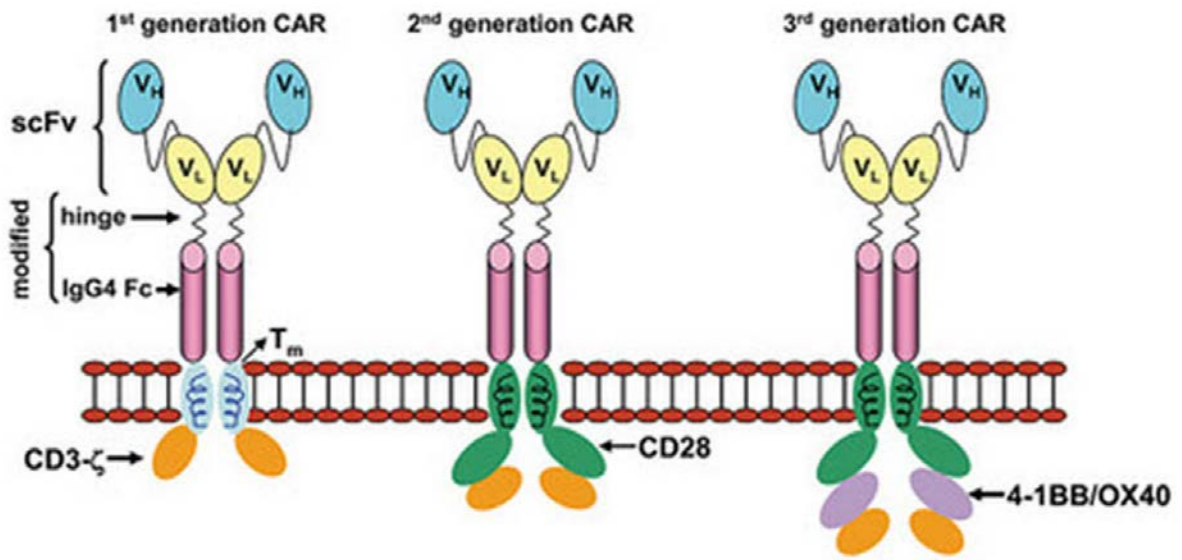

Figure 3. Schematics of prototypical CARs used to redirect T cell specificity. CARs exist in a dimerized form and are expressed as a fusion protein that links the extracellular scFv (V_H linked to V_L) region, a flexible hinge, an Fc region such as IgG4, a transmembrane domain, and intracellular signaling motifs. The endodomain of the 1st generation CAR induces T cell activation solely through CD3-ζ signaling. The second generation CAR provides activation signaling through CD3-ζ and CD28, or other endodomains such as 4-1BB or OX40. The 3rd generation CAR activates T cells via a CD3-ζ-containing combination of three signaling motifs such as CD28, 4-1BB, or OX40.

Adapted from: Kebriaei P *et al*. 2012. CARs: Driving T-cell specificity to enhance anti-tumor immunity. *Frontiers in Bioscience* 4:520-31. With permission from Frontiers in Bioscience.

1.5 Improving Adoptively Transferred T Cell Persistence

Despite the design of the 1st generation CARs to mimic T cell receptor (TCR) induction by signaling via a CD3ζ endodomain to activate modified-T cells, this did not provide the fully competent signal that T cells receive in the physiological context. It has been revealed that 1st generation CARs could induce proliferation for a few cycles but T cell death quickly ensued (44). Normally when T cells are activated *in vivo* they receive a primary antigen-induced TCR signal with secondary costimulatory signaling from CD28 that induces the production of cytokines (i.e., IL-2 and IL-21), which then feed back into the signaling loop in an autocrine/paracrine fashion (45-47). With this in mind, 2nd generation CARs were constructed to include CD28 cytoplasmic signaling domain (41) or other costimulatory molecule signaling domains such as 4-1BB (48)(**Fig. 3**). Chimeric CD28 co-stimulation improves T-cell persistence over the 1st generation CAR by up-regulation of anti-apoptotic molecules and production of IL-2, as well as expanding T cells derived from peripheral blood mononuclear cells (PBMC) (41). Though T cell survival is enhanced, murine experiments demonstrated a significant decline in 2nd generation CAR T cell persistence within the first seven days after adoptive transfer (41), indicating key supportive factors are still missing.

Efforts are still underway to identify the optimal combination of signaling endodomains and to develop new strategies to further enhance *in vivo* survival of CAR T cells. The continued development in CAR technology has led to the construction of 3rd generation CARs that create triple endodomain fusion receptors and appear to improve *in vitro* function in comparison to the 2nd generation CAR (49) (**Fig. 3**). Other reported approaches that demonstrated enhanced T cell persistence in adoptive transfers are: (i) the infusion of effector $CD8^+$ T cells derived from central memory (T_{CM}), naïve (T_N), and memory stem cell (T_{SCM}), which demonstrated progressively increasing therapeutic potential (50-52); (ii) generation of bi-specific T cells with tumor antigen recognition via an introduced CAR and viral antigen specificity via the endogenous TCR that provided a more competent activation signal (39); *ex vivo* propagating CAR T cells in specific cytokine cocktails (53, 54); and (iv) the expression of the costimulatory molecules CD80 and 4-1BBL on T cells that provide auto- and trans-costimulatory signals (42).

While each of these approaches have significant implications for the generation of cells for adoptive immunotherapy, continued development of new strategies to enhance modified T

cell persistence is still necessary as the described advances that have been made either: have not yet demonstrated robust long-term persistence in humans (50-52), may not be appropriate across clinical platforms of immunotherapy (39), or persisted only in a tumor associated antigen (TAA)-dependent manner (42). Thus, alternate means for improving T-cell persistence is warranted and the specific aims of this dissertation address this.

1.6 Cytokines Support Adoptively Transferred T Cell Persistence

Cytokines in the common gamma chain receptor (γc) family collectively share a four α-helical bundle structure which signal through hetero-dimeric and trimeric receptor complexes that share the γc sub-unit (reviewed in reference (55)). Interleukin (IL)-2, IL-7, IL-15, and IL-21 are members of this family and signal through Janus family tyrosine kinase (JAK)1 and JAK3. IL-2, IL-7, and IL-15 primarily activate both signal transducer and activator of transcription (STAT)5, whereas IL-21 mainly activates STAT3 (56). Cross-talk also occurs with the Ras, PI3K (57), and nuclear factor-κB (NF-κB) (58) pathways. While the shared signaling of the γc may contribute to the overlapping functions of these cytokines, signaling of their private receptors as well as coupling with other signaling pathways lends to the unique and pleiotropic effects of these cytokines (57).

IL-2 has been the cytokine historically used in the clinic to improve transferred T cell persistence. IL-2 signals through a low affinity state via a hetero-dimeric receptor with IL-2/IL-15 receptor beta chain (IL-2/IL-15Rβ; also known as CD122) and γ_C subunits. High affinity signaling is enabled by IL-2 receptor alpha chain (IL-2Rα) when it is present in the heterotrimeric receptor complex with IL-2Rβ and γc subunits (59). IL-2 is a growth factor that promotes T cell activation and proliferation (60) while also being important for the differentiation of T cells to the T_H2 phenotype (61). The administration of IL-2 is used to boost T cell survival *in vivo* during adoptive therapy (62, 63). However, prolonged systemic dosage of IL-2 is associated with severe toxicities including respiratory distress and vascular leak syndrome, resulting in limits to IL-2 usage in the treatment (62, 64). Additionally, IL-2 can even hinder the effectiveness of infused T cells via the expansion of regulatory T cells that induce peripheral tolerance (65) and the promotion of activation-induced cell death (AICD) (66). The focus is now shifting to investigating the other γc cytokines to (i) generate higher quality T cells *in vitro* and (ii) to better support adoptively transferred T cells *in vivo*.

IL-15 has leapt into the spotlight in the past few years due to its potential for use in the immunotherapy field. It is a homeostatic cytokine that appears to exert a preferential effect on the homeostatic proliferation of CD8 memory T cells (67, 68). It has also been reported that IL-15 can induce rapid homeostatic proliferation of naïve T cells in lymphopenic conditions, with the resultant expanded cells possessing central memory characteristics (69). Moreover, it was observed that *in vitro* culture with low-dose IL-15 resulted in proliferation without phenotypic change (67), which is an attribute of great potential with regard to following the paradigm shift toward generating T cells with maximal memory potential.

IL-15 has been established to function *in vivo* by a transpresentation mechanism. Activated monocytes or dendritic cells present a membrane-bound IL-15 receptor alpha chain (IL-15Rα)/IL-15 complex to the IL-2/IL-15Rβ and γc receptor complex on the T cell (70) (**Fig. 4**). IL-15Rα is the high affinity receptor that binds IL-15 resulting in qualitatively different signal transduction than that achieved by soluble IL-15 (71). In addition, the IL-15/IL-15Rα complex undergoes endosomal recycling back to the surface thereby extending the cytokine's persistence in the system (70, 72) (**Fig. 4**). Interestingly, the transpresentation of IL-15 occurs in the immunological synapse. It is speculated that this severely restricts IL-15 exposure to prevent unintended immune stimulation (73).

Consideration of the transpresentation mechanism is important in harnessing the cytokine's potency and biological activity. The use of IL-15/IL15Rα complexes resulted in increased potency over the soluble cytokine and was able to promote the eradication of established tumors via alleviating functional suppression of tumor-resident cells (74). A non-human primate study indicated that IL-15 was far superior to IL-2 in generating long-lived memory T cells (75). In addition, safety has been evaluated in non-human primate models. An intermittent dosing schedule was found to be well tolerated, and it was observed that memory CD8 and CD4 T cells as well as NK cells were detected in the peripheral blood in this lymphoreplete model (76). The potential for IL-15 in modulating cellular responses in ACT therapies has excited the field and numerous clinical trials have been initiated and are rapidly gaining momentum. <u>The demonstrated biological responses of IL-15 make it an excellent candidate for incorporation into our CAR T cell therapy treating ALL MRD.</u>

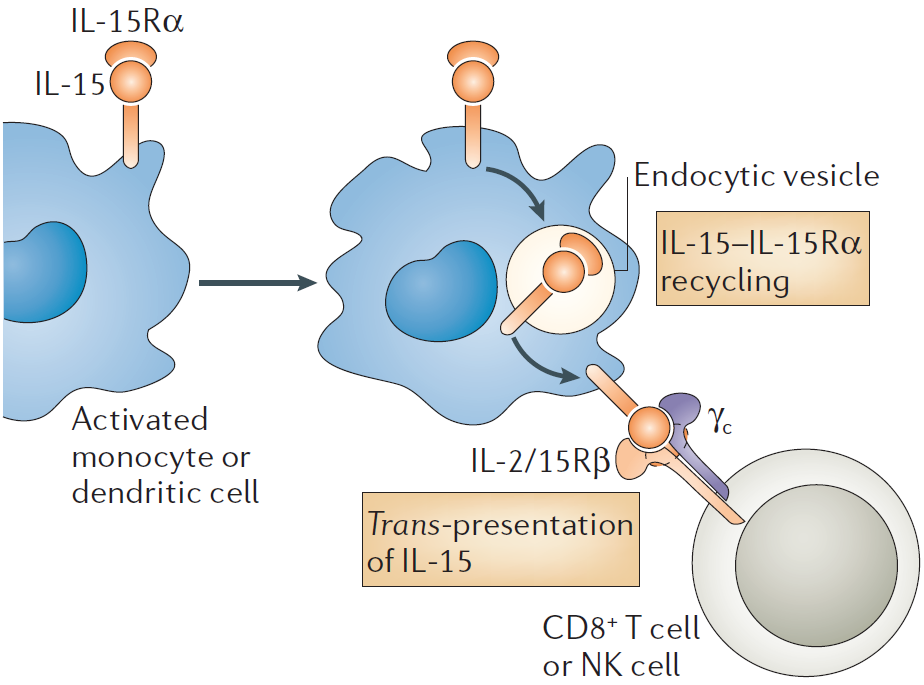

Figure 4. Transpresentation of IL-15 by IL-15Rα to neighboring CD8⁺ T cells. Type I or type II interferons induce coordinate expression of IL-15 and the IL-15Rα on monocytes or dendritic cells. The IL-15/IL-15Rα complex recycles from the cell surface through endosomal vesicles to be re-expressed at the cell surface. IL-15 is presented in *trans* by IL-15Rα to T cells expressing the IL-2/15Rβ and γ_c receptor complex.

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1.7 Specific Aims

The primary goal of this dissertation is translationally driven and seeks to develop an alternative approach to address a clinical need and contribute to furthering the therapeutic potential of adoptive cell therapy (ACT). Three specific aims are pursued that bring forth novel information or improve upon an immunotherapeutic approach contributing to the above gaps in knowledge or prevailing clinical issues. There is consensus that the persistence of adoptively transferred CAR T cells remains inconsistent and impacts the therapeutic efficacy of ACT. More specifically, persistence of transferred T cells may pose to be more challenging in low or sequestered TAA environments. With that clinical context in mind, our approach capitalized on the pleiotropic effects and unique mechanistic features of IL-15 to develop a membrane-bound IL-15/IL-15Rα fusion protein **(mIL15)** to be co-expressed on CAR T cells. The **overarching hypothesis** of this dissertation is that mIL15 sustains costimulation to improve CAR T cell persistence and antitumor activity whilst preserving memory potential.

Specific Aim 1: IL-15 mediates numerous immunomodulatory effects that are beneficial for ACT, including supporting the homeostasis of long-lived T_{CM}, and is therefore a costimulatory molecule suitable for improving the persistence of CAR T cells. Incorporating the prevailing *in vivo* mechanistic action of IL-15, we generated mIL15. In this specific aim we **hypothesize** that mIL15 can sustain STAT5 signaling to costimulate CAR T cells by mimicking transpresentation. Additionally, as part of the translational aspect of this project, we aimed to generate sufficient numbers of functional and clinically relevant mIL15-CAR T cells. Using a clinical platform to genetically modify and expand mIL15-CAR T cells, these cells will be evaluated by: (i) phosflow to assess the mIL15 costimulatory effect via pSTAT5 induction (ii) extended phenotyping for T cell and memory-associated markers using flow cytometry, and (iii) assess redirected specificity by a chromium release assay (CRA) and IFNγ production in response to $CD19^{neg}$ and $CD19^{+}$ targets.

Specific Aim 2: IL-15 can enhance *in vivo* antitumor activity and support homeostasis of CD8 T_{CM}, which persist long-term without requiring contact with antigen. We **hypothesize**, therefore, that mIL15-CAR T cells will possess enhanced antitumor activity *in vivo* and persist *in vivo* independently of TAA. Immunocompromised mice will be used to assess mIL15-CAR T cell performance (antitumor activity and/or T cell persistence) under three

models: (i) a high-dose leukemia xenograft, (ii) a preventative low-dose disseminated leukemia xenograft, and (iii) T cell engraftment in the absence of TAA.

Specific Aim 3: The differentiation fate of long-term persisting cytokine and CAR co-modified T cells is ambiguous and it is unknown whether intrinsic factors play a role in addition to the extrinsic elements. IL-15 plays a critical role in the homeostatic maintenance of CD8 T_{CM}. We therefore **hypothesize** that persisting mIL15-CAR T cells will possess attributes associated with T_{CM}. Moreover, intrinsic elements will be representative of their differentiation state. We sought to characterize long-term persisting mIL15-CAR T cells by first modeling the persistence *in vitro* to assess: (i) mIL15 and CAR phenotype by flow cytometry, (ii) mIL15 functionality by phosflow and functionality of CAR by IFNγ production assessed by flow cytometry, (iii) gene expression analysis by nCounter, (iv) phenotyping of transcription factors and surface markers associated with T cell differentiation state, (v) memory-associated function via assessment of IL-2 production, and (vi) CCR7 subset viability by Annexin V staining. *In vivo* assessments followed up by assessing mIL15-CAR T cells persisting in immunocompromised mice in the absence of TAA and evaluated: (i) phenotyping of surface markers associated with T cell differentiation state, (ii) mIL15 and CAR phenotype, and (iii) CAR functionality by IFNγ production in response to $CD19^{+/-}$ targets assessed by flow cytometry.

CHAPTER 2

Generation of Clinically Relevant CD19-specific T Cells Co-expressing mIL15

2.1 Introduction

CARs are used to retarget T cell specificity to a desired surface expressed TAA (37). Although adoptive transfer of CAR T cells have shown objective clinical responses in treating B cell leukemias, successful outcomes in adoptive immunotherapy are associated with persistence of transferred antigen-specific T cells (43, 63, 77, 78). CAR-modified T cells are designed to receive costimulation for activation, survival, and proliferative signaling via the introduced immunoreceptor when engaged with a TAA (79). This approach, even with the addition of other costimulatory molecules, predictably lead to *in vivo* accumulation of T cells only in a TAA-dependent manner (41, 42). In low level TAA environments, infused T cells may not receive sufficient CAR-mediated signaling for robust persistence. This raises concerns for their capacity to persist at therapeutic levels to mediate durable tumor surveillance in leukemic relapse prevention or to treat MRD. Clinical trials will now be increasingly targeting the treatment of MRD as patients are enrolled earlier in their disease course and are receiving concomitant chemotherapy. Thus, optimizing adoptively transferred T cell persistence under low TAA conditions is paramount to improving clinical response and this aspect remains aggressively pursued.

Administration of exogenous interleukin IL-2 has been the cytokine historically relied upon to enhance survival of adoptively transferred T cells (62, 63). Systemic high-dose IL-2 has proven to potentiate dose-limiting toxicities that include respiratory distress and vascular leak syndrome (62, 64, 80), as well as risks AICD that may limit the therapeutic response of transferred T cells (81, 82). While low-dose IL-2 has been shown to be better tolerated, it appears to contribute to peripheral tolerance and the production of regulatory T cells (65, 83, 84). IL-2 is also used in the *in vitro* expansion of T cells for adoptive therapy and is associated with inducing a differentiated effector phenotype (53), which have been demonstrated to have far poorer *in vivo* persistence and therapeutic potential than other less differentiated T cell subsets (52). Contrasting the detrimental biological effects of IL-2, IL-15 possesses a plethora of immunomodulatory attributes desirable to adoptive therapy.

It is a pro-survival cytokine required for the homeostatic maintenance of long-lived CD8 memory T cells (85, 86), inhibits AICD (87), enhances *in vivo* antitumor activity (88), and reverses T cell anergy (89). With this valuable arsenal, IL-15 has been ranked the most important immunotherapeutic agent for cancer treatment (90) and has been used in metastatic melanoma and renal carcinoma trials with recently registered trials with natural killer (NK) cell and T cell immunotherapies (www.clinicaltrials.gov: NCT01385423, NCT01369888, NCT01875601).

Clinical trials, thus far, have administered soluble recombinant IL-15 and pre-clinical studies have engineered antigen-specific human T cells that secrete monomeric IL-15 (91-93). However, unlike IL-2 which is secreted, IL-15 is uniquely transpresented in the context of IL-15Rα to responding T cells by the producing cells (i.e., dendritic cells) (94, 95). Limitations exist to the use of monomeric IL-15 as it is an unstable low molecular weight protein resulting in a short serum half-life thus necessitating frequent supra-physiologic dosing to attain biological responses *in vivo* (76, 96, 97). Mimicking physiologic transpresentation by coupling IL-15 with a soluble form of IL-15Rα remedies this shortcoming and has shown greater potency and antitumor efficacy of T cells *in vivo* (97), likely due in part to the qualitatively different signaling achieved by transpresentation which result in more robust activation of the cells (71). Moreover, it was demonstrated that targeting complexed IL-15 to the tumor microenvironment, and thus to responding immune cells, further enhanced the antitumor effect (98).

The ***hypothesis*** of this chapter is that expression of mIL15 on CAR T cells will function by the physiologic mechanism of transpresentation and will induce enhanced STAT5 costimulation. This work aims to generate pre-clinical data for the development of a Phase I clinical trial testing the therapeutic efficacy of mIL15-CAR T cells in treating MRD and is therefore also focused on generating mIL15-CAR T cells that can meet specific clinically relevant criteria, including generating sufficient numbers of modified T cells, retaining redirected specificity, and a quality phenotype that includes the expression of memory–associated surface markers. Here we show the co-modification of CAR T cells with mIL15 in primary human cells that are independent of exogenous cytokine stimulation with the generated mIL15-CAR T cells suitable for adoptive transfer.

2.2 Results

2.2.1 mIL15 Plasmid Construction

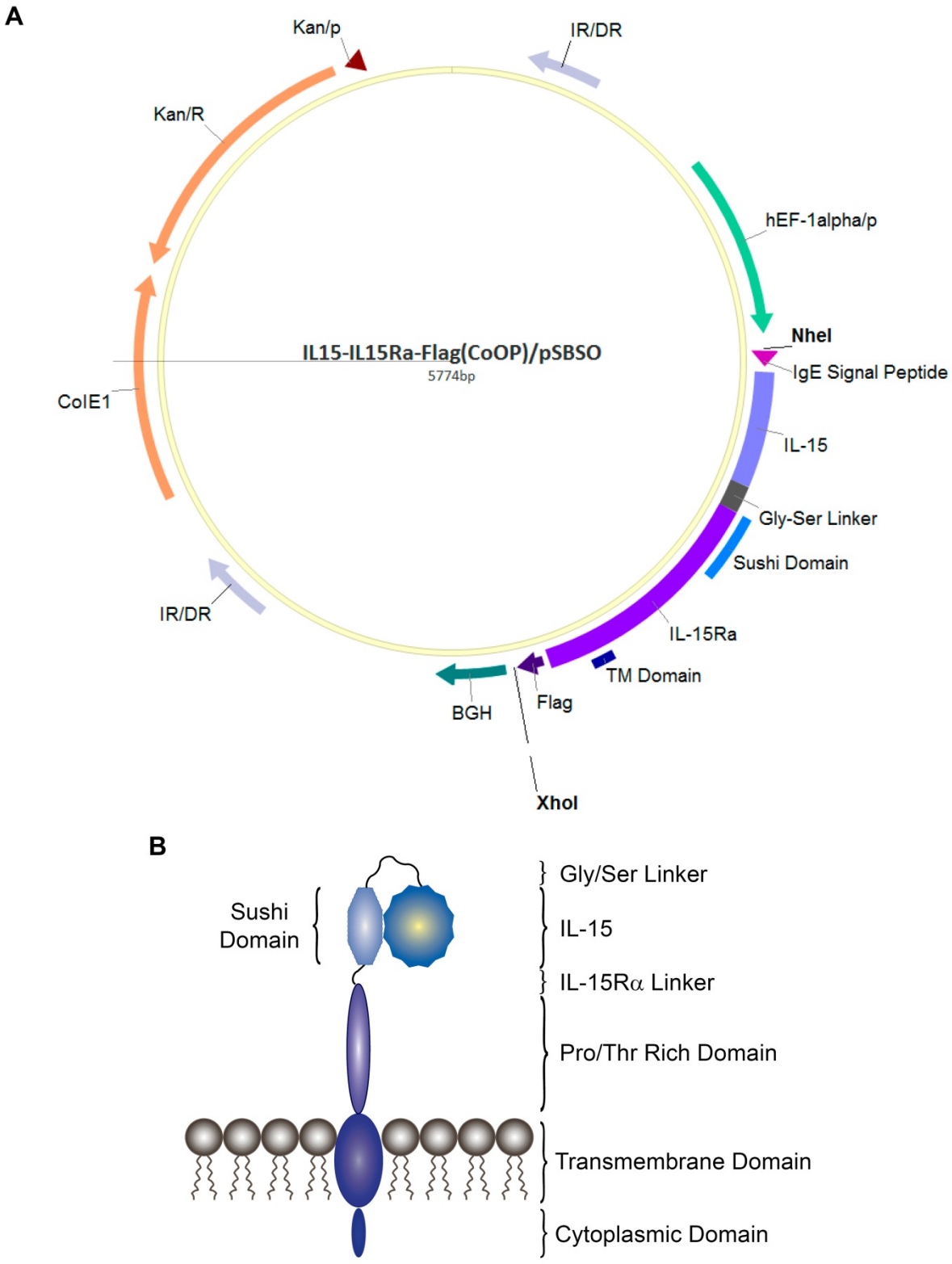
To provide human primary T cells with self-sustained IL-15 signaling that mimics the primary *in vivo* mechanism of action of transpresentation, we designed a tethered IL-15 fusion protein to be expressed on the surface of the CAR T cell. The expression of IL-15 is highly regulated at multiple levels, but with several critical elements functioning at the post-translational level, including the signal peptide and coding sequence (99). Alternate codon usage and signal peptides have been shown to increase IL-15 expression levels (91, 100). The mIL15 construct is codon-optimized and consists of a fusion of IL-15 cDNA (NM_000585.4) to the full length IL-15Rα (NM_002189.3) with a flexible linker and is inserted within a S*leeping Beauty* (*SB*) expression plasmid. The signal peptides for IL-15 and IL-15Rα were omitted and the IgE signal peptide (gb|AAB59424.1) was used for the mIL15 construct (**Fig. 5A**). The mIL15 DNA sequence was codon optimized and synthesized by GeneArt (Regensburg, Germany) and subsequently subcloned into a *SB* transposon plasmid. *SB* transposons are used as part of a non-viral gene transfer approach that is directly translatable to the clinic as it is used in some CAR T cell clinical trials for genetic modification of the T cells (IND#s: 11470, 14577, 14739) (101, 102). Translation of this tethered IL-15 fusion protein is expected to synthesize a membrane-bound IL-15 that, based on similar molecules (103, 104), is expected to emulate presentation of IL-15 in context with IL-15Rα (**Fig. 5B**).

2.2.2 Generation and Expression of mIL15 and CAR on Primary Human T Cells

To keep with the direct translational applicability of this study, the genetically modified T cells were generated and *ex vivo* propagated using clinically compliant methods (**Fig. 6**) (102, 105). Primary normal donor human T cells were co-electroporated with a CD19-specific CAR transposon, with or without the mIL15 transposon, and the SB-11 transposase to generate mIL15-CAR T cells and the conventional CAR T cells to serve as a clinically relevant comparison. The CAR used was '2nd generation', thus comprised of CD3ζ and CD28 signaling endodomains (41). Propagation of the genetically modified T cells was achieved by recursive stimulation (Stim) with a K562 artificial antigen presenting cell line (aAPC) variant expressing CD19 antigen, as well as 41BBL and CD86 co-stimulatory molecules. The presence of CD19 on the aAPC allows for the selective outgrowth of antigen-specific T cells while the costimulatory molecules improve *in vitro* expansion (106).

Figure 5. Schematics of mIL15. (A) *Sleeping Beauty* DNA plasmid map for mIL15 [IL15-IL15Ra-Flag(Coop)/pSBSO] for the expression of a fusion protein consisting of IL-15 linked with the full-length IL-15Rα. (B) A schematic representing the protein structure of mIL15 consisting of IL-15 linked with the full-length IL-15Rα. Abbreviations: hEF-1alpha/p, human elongation factor-1 alpha promoter; TM, transmembrane domain, BGH, polyadenylation signal from bovine growth hormone; IR/DR, *Sleeping Beauty* inverted repeat; ColE1, *E. coli* origin of replication; Kan/R, gene encoding kanamycin resistance for bacterial selection; Kan/p, prokaryotic promoter.

Figure 5. Schematics of mIL15



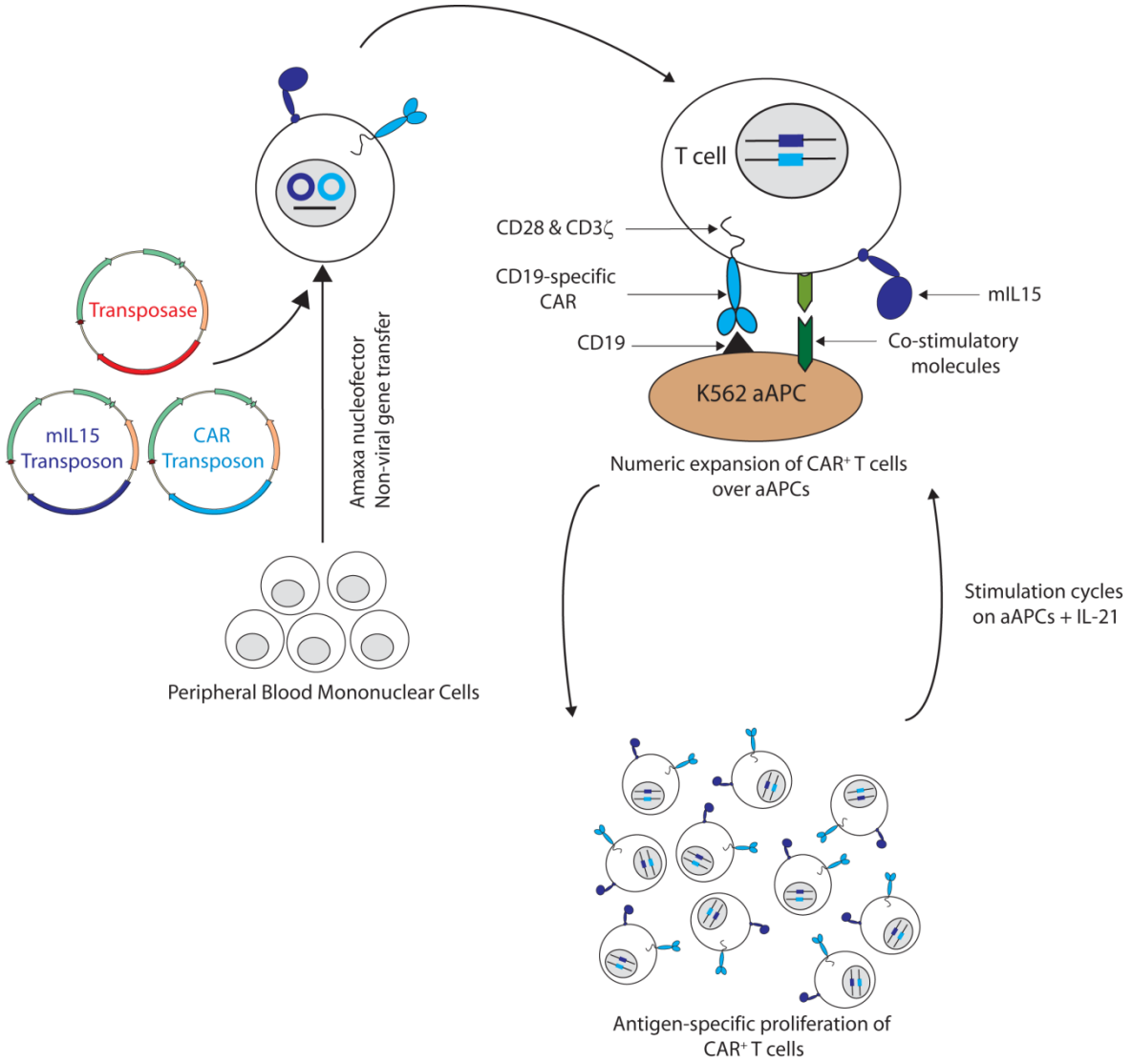


Figure 6. Generation and *ex vivo* expansion of mIL15-CAR T cells using a clinical platform. Schematic of the clinically compliant methods adopted for generating and numerically expanding mIL15 and CAR co-modified T cells. PBMC were electroporated with mIL15 and CAR *Sleeping Beauty* (*SB*) DNA transposons and *SB* transposase. Cells were then cocultured with γ-irradiated K562 (Clone 9)-derived aAPC (modified to express CD19, CD64, CD86, and CD137L) and, unless otherwise stated, had supplementation with IL-21 (30ng/ml). The genetic modification and expansion of CAR T cells was similarly performed, but using only the CAR *SB* transposon and *SB* transposase during electroporation with subsequent cultures supplemented with IL-21 (30ng/ml) and IL-2 (50U/ml).

Transient transfection levels of CAR were similar between CAR and mIL15-CAR T cells. The mIL15 was detected at modest levels in mIL15-CAR T cells (**Fig. 7A**). After four Stims, nearly all T cells expressed CAR and expression was equivalent between CAR and mIL15-CAR T cells (**Fig. 7B**). The mIL15 molecule was stably co-expressed with the CAR at 51 ± 14% (mean ± SD), and a minimal expression of mIL15 without the CAR was observed (2 ± 4%; mean ± SD; **Fig. 7A,C**). CAR mean fluorescence intensity (MFI) was equivalent between CAR T cells and $mIL15^{+}CAR^{+}$ T cells. However, $mIL15^{neg}CAR^{+}$ T cells showed significantly lower CAR MFI compared to $mIL15^{+}CAR^{+}$ T cells ($P < 0.001$; **Fig. 7D**), but the functional implications remain unknown. These data show that mIL15 and CAR can be stably co-expressed in primary T cells.

2.2.3 Numeric Expansion of Clinically Significant Numbers of mIL15-CAR T Cells

After redirecting T cell specificity with the CAR, suitable numbers of these modified T cells must be generated to allow for adoptive transfer of therapeutic doses of CAR T cells. To assess whether mIL15 could provide enhanced costimulation sufficient for sustaining *in vitro* numeric expansion, mIL15-CAR T cells were propagated as previously described but without exogenously added IL-21 (**Fig. 6**) and compared to CAR T cells that received (i) no cytokine supplementation (baseline growth control), (ii) IL-15 complex (soluble cytokine control), or (iii) IL-2 and IL-21 (clinical or positive control). Unlike CAR T cells that received no cytokine supplementation, numeric expansion of mIL15-CAR T cells was achieved in the absence of exogenous cytokine supplementation ($P < 0.0001$; **Fig. 8A**). And, while mIL15-CAR T cell expansion was equivalent to CAR T cells supplemented with IL-15 complex, these cells could not parallel the expansion of conventional CAR T cells supplemented with IL-2 and IL-21 ($P < 0.0001$; **Fig. 8A**). This may have been due, in part, to the persistent overgrowth of NK cells at early time points (Stim 2) which was observed to comprise greater than 70% of the cells in the culture but was not an issue in conventional CAR T cell cultures ($P < 0.01$; **Fig. 8B,C**). Despite CD56 depletion of mIL15-CAR T cell cultures, phenotyping after *ex vivo* expansion (Stim 4) revealed moderate levels of $CD3^{neg}CD56^{+}$ and $CD3^{neg}CD56^{neg}$ populations in T cell cultures. These non-T cell subsets cumulatively comprised 19% - 55% of the culture and a concomitant decrease in the outgrowth of desirable $CD8^{+}$ T cells for adoptive transfer was observed (**Fig. 8D**). IL-15 may have played a role in supporting the growth of the non-T cell subsets, as CAR T cell cultures supplemented with IL-15 complex also showed these subsets cumulatively comprise up to 86% of the cells in culture (**Fig. 8D**).

Figure 7. Stable co-expression of mIL15 and CAR on T cells after propagation on aAPC. (A) Transient (24-hours post-electroporation) and stable (Stim 4) expression of mIL15 and CAR on *Sleeping Beauty*-modified T cells. A representative flow plot is shown for one of 10 normal donors. Gate frequencies are displayed in the top right corner of the plot. (B) Frequency of expression of CAR on CAR T cells and mIL15-CAR T cells. Horizontal bar represents the mean. (C) Frequencies of mIL15 and/or CAR expressing subsets of mIL15-CAR T cells. Data are mean ± SD, n = 4. (D) The CAR mean fluorescence intensity (MFI) on CAR and mIL15-CAR T cells. The mIL15-CAR T cells were sub-divided based on their mIL15 expression. ***P < 0.001, ns = not significant, paired t test (B), one-way ANOVA (C).

Figure 7. Stable co-expression of mIL15 and CAR on T cells after propagation on aAPC

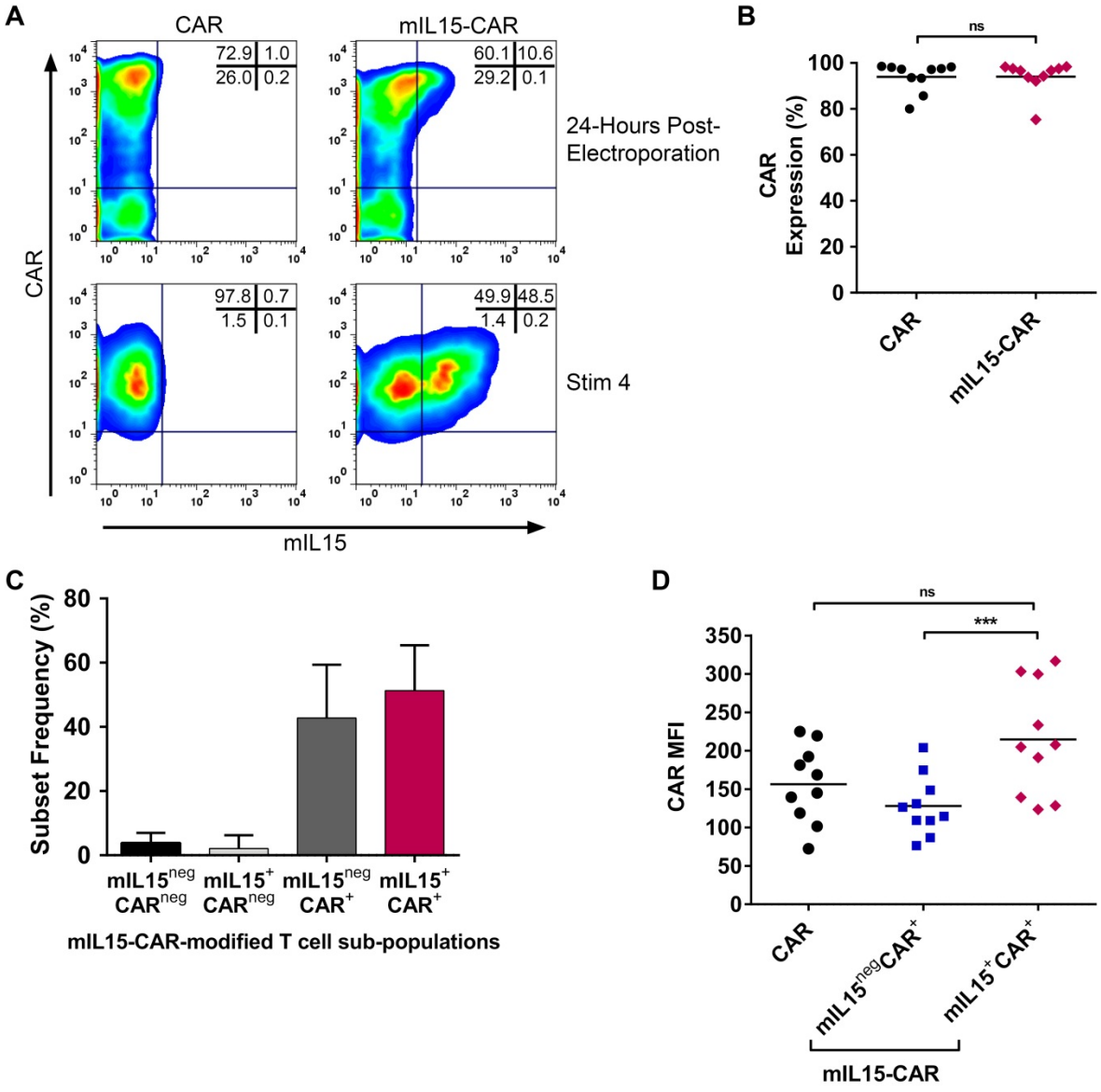
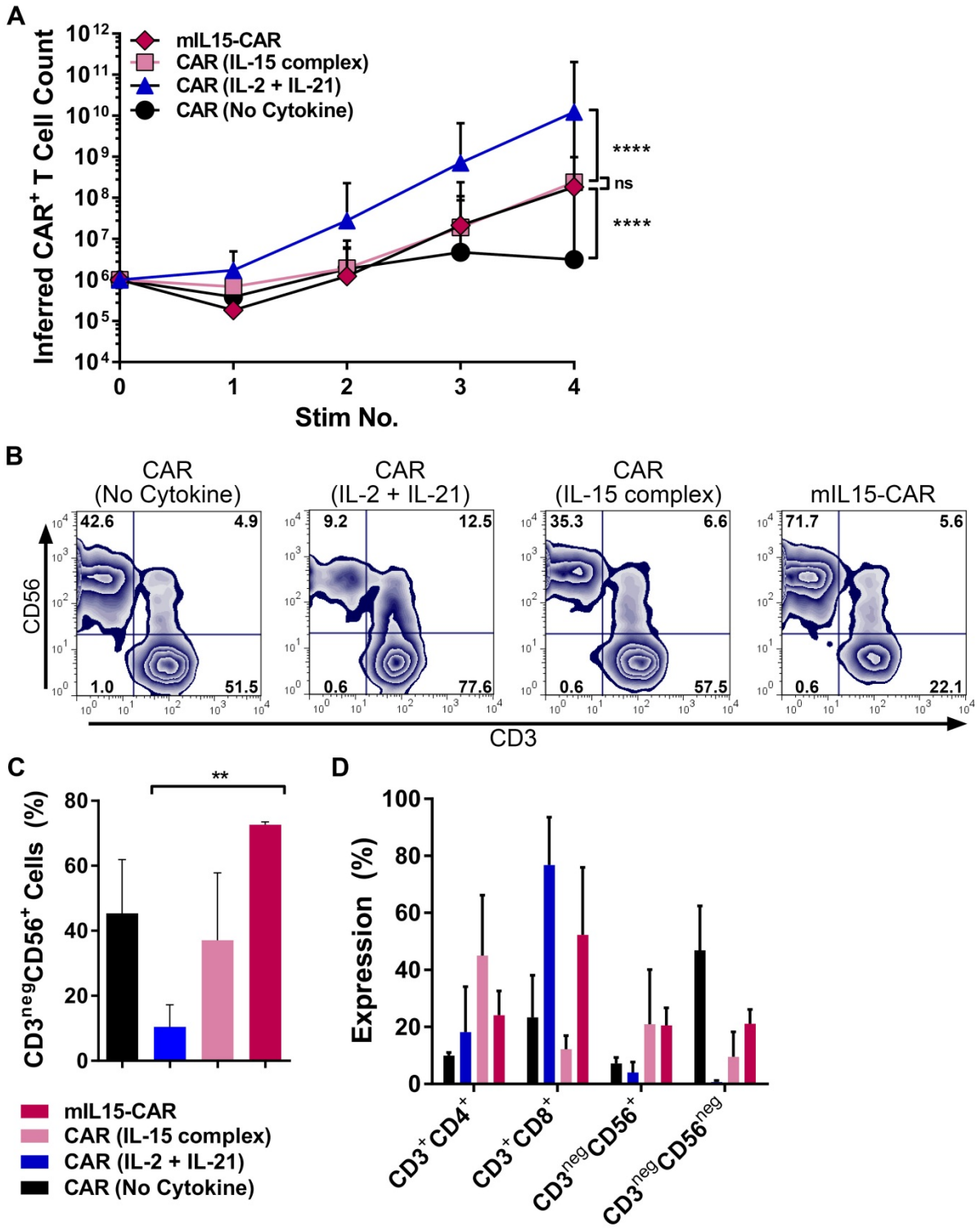


Figure 8. Numerical expansion of mIL15-CAR T cells on aAPC without exogenous cytokines is accompanied by an NK cell expansion. (A) Rate of CAR-specific expansion of mIL15-CAR T cells with no exogenous cytokine administration and CAR T cells supplemented with IL-2 (50U/ml) and IL-21(30ng/ml), IL-15 complex (5ng/ml), or unsupplemented. At Stim 2, cultures containing > 15% $CD3^{-}CD56^{+}$ cells were paramagnetic bead sorted to deplete $CD56^{+}$ cells. Data are expressed as mean ± SD, *n* = 6. ****P < 0.0001, ns = not significant, two-way RM ANOVA (Tukey's post-test). (B) Expression of CD3 and CD56 on cells at Stim 2. Representative flow plots are shown for one of three normal donors. (C) Frequencies of $CD3^{-}CD56^{+}$ NK cells detected in cultures at Stim 2 from (b). Data are expressed as mean ± SD, *n* = 3. **P < 0.01, one-way ANOVA (Tukey's post-test). (D) Frequencies of T cell sub-populations co-expressing combinations of CD3, CD4, CD8, or CD56. Data are expressed as mean ± SD, *n* = 3.

Figure 8. Numerical expansion of mIL15-CAR T cells on aAPC without exogenous cytokines is accompanied by an NK cell expansion



In generating CAR T cells for adoptive therapy, the focus in *ex vivo* propagation is on driving high numeric expansion of CAR^+ T cells. In accordance with GMP protocols that yield high numbers of CAR T cells (102), the conventional CAR T cell cultures were supplemented with IL-2 and IL-21. This study capitalized on the reported synergistic proliferative effect of IL-21 with IL-15 on $CD8^+$ T cells (107) to increase expansion of $CD8^+$ mIL15-CAR T cells. With IL-21 supplementation to the culture protocol, mIL15-CAR T cells demonstrated comparable expansion to conventional CAR T cells, yielding $2.3 \times 10^7 – 7.4 \times 10^{10}$ and $6.1 \times 10^8 – 4.4 \times 10^{10}$ CAR^+ T cells, respectively (**Fig. 9**). In sum, while mIL15 may provide some costimulatory support during *ex vivo* expansion, IL-21 is a necessary addition to enable the generation of clinically relevant numbers of mIL15-CAR T cells.

2.2.4 Enhanced costimulation of mIL15-CAR T Cells Via pSTAT5 Induction

Additional investigation of the costimulatory capability of mIL15 was performed by phosflow assessing phosphorylated signal transducer and activator of transcription 5 (pSTAT5), the primary signaling molecule involved in IL-15 receptor complex signal transduction (108). Unlike cytokine unsupplemented CAR T cells, mIL15-CAR T cells (receiving no exogenous cytokines) demonstrated sustained pSTAT5 levels ($P < 0.01$; **Fig. 10A,B**). Moreover, the costimulation intensity was below that induced by the supra-physiologic dosing of soluble IL-15 in CAR T cells ($P < 0.05$; **Fig. 10B**), suggesting that the phosphorylation of STAT5 molecules was not saturated and thus mIL15 may induce more of a physiologically relevant signal strength.

A primary reason for designing mIL15 as membrane-bound and with IL-15Rα, was to mimic the mechanism of IL-15 transpresentation that occurs *in vivo*. To assess whether mIL15 can transpresent IL-15, a co-culture experiment was performed where CAR and mIL15-CAR T cells were co-cultured at a ratio greater than 1:4 and at high density to ensure cell-cell contact. IL-15-specific or Flag antibodies were used to distinguish the two populations. Unlike CAR T cells cultured alone, those in co-culture with mIL15-CAR T cells were able to receive IL-15 signal induction in *trans*, as indicated by sustained pSTAT5 levels (**Fig. 10C**). Together these data show that mIL15 provided sustained costimulation that induced STAT5 signal transduction and that this costimulation could be initiated in *trans* to mimic physiologic mechanisms.

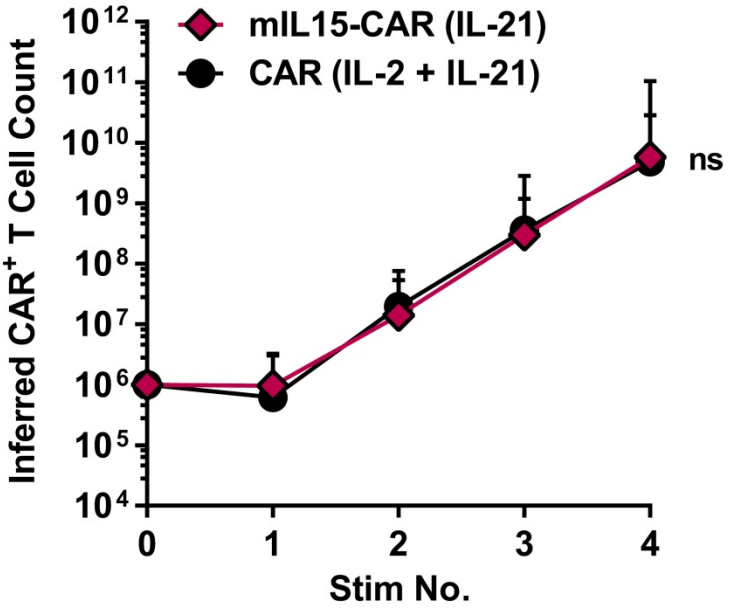


Figure 9. Numerical expansion of mIL15-CAR T cells on aAPC with IL-21 supplementation. Rate of CAR-specific T cell expansion of mIL15-CAR T cells cultured with exogenous IL-21 (30ng/ml) and CAR T cells supplemented with IL-2 (50U/ml) and IL-21(30ng/ml). Cultures exceeding > 15% $CD3^{-}CD56^{+}$ cells were paramagnetic bead sorted to deplete $CD56^{+}$ cells. Data are expressed as mean ± SD, *n* = 6, ns = not significant, two-way RM ANOVA (Bonferroni post-test).

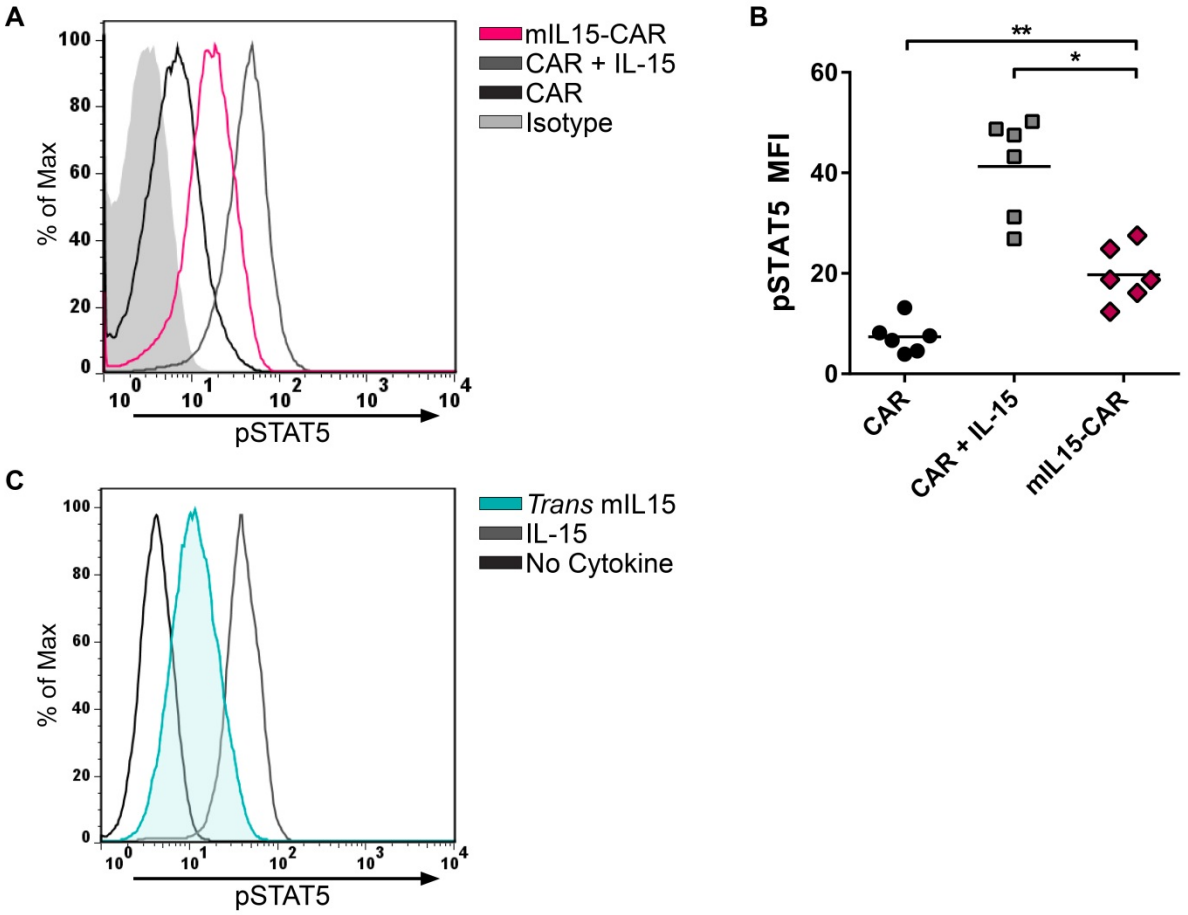


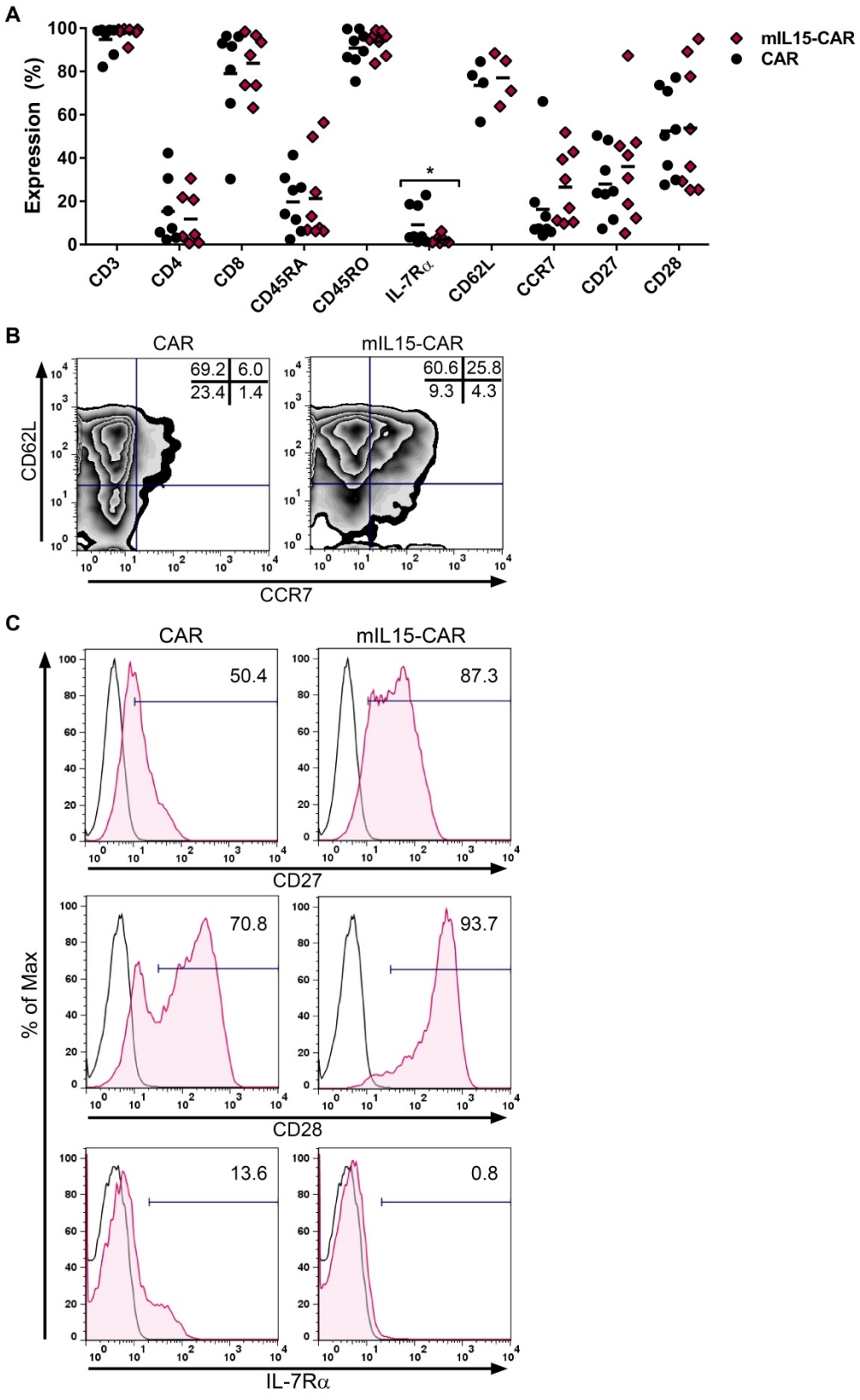
Figure 10. Induction of pSTAT5 by mIL15. Functionality of mIL15 was validated by phosflow of pSTAT5 in CAR and mIL15-CAR T cells under serum and cytokine starved conditions. (A) A representative histogram showing the flourescence intensity of pSTAT5 in CAR and mIL15-CAR T cells (n = 6). CAR T cells treated with soluble IL-15 (100ng/ml) for 1-hour prior to fixation served as the positive control whereas unsupplemented CAR T cells indicate baseline pSTAT5 levels. (B) Mean flourescence intensity (MFI) of pSTAT5 of modified T cells from (A). Horizontal bar represents the mean. *P < 0.05, **P < 0.01, one-way ANOVA. (C) The representative historgram shows pSTAT5 flourescence intensity of CAR T cells in co-culture with mIL15-CAR T cells (transpresented mIL15) at a ratio of 1:4, CAR T cells supplemented with IL-15, and unsupplemented CAR T cells (n = 4). Histogram is gated on IL-15^{neg} cells.

2.2.5 Immunophenotype and Function of mIL15-CAR T Cells is Unaltered by mIL15

Concerns have arisen that T cells generated for adoptive therapy undergo excessive culture time, excessively driven expansion, or exposure to cytokines such as IL-2 that promote T cell differentiation toward terminal effector cells, thereby diminishing antigen-specific T cell therapeutic potential (17, 109, 110). For that reason expanded CAR T cells, also considered the infusion product for adoptive transfers, are assessed for surface markers that may indicate the cell product's *in vivo* or clinical potential. The immunophenotype of CAR and mIL15-CAR T cells was not significantly altered by mIL15 expression. CAR and mIL15-CAR T cells nearly all expressed CD3, CD8, and CD45RO. CAR and mIL15-CAR T cells expressed: CD62L ranging from 57% - 85% and 64% - 88%; CCR7 ranging from 3% - 31% and 7% - 31%, CD27 ranging from 7% - 50% and 5% - 87%; and CD28 ranging from 28% - 77% and 25% - 95%, respectively (**Fig. 11A-C**). Only a low level of IL-7Rα expression by mIL15-CAR T cells was noted ($P = 0.0470$). The moderate to high expression levels of various memory-associated markers (17, 18, 21, 111) for both sets of modified T cells indicates a heterogeneous mix of T cell differentiation subsets. There is no indication of dominance of the potentially more therapeutically limited terminally differentiated effector or effector memory subsets, which would be characterized by a lack of CD62L and CCR7. To the contrary, these modified T cells display characteristics predictive of *in vivo* persistence and treatment responses (52, 112-114). After *ex vivo* expansion of the modified T cells, it is important to validate, for clinical purposes, that the modified T cells retain their redirected T cell specificity toward CD19. A chromium release assay was conducted to assess the function of the T cells in the expansion product. $CD19^{+}$ and $CD19^{neg}$ EL4 targets were plated with CAR and mIL15-CAR T cells at across a range effector to target ratios. As expected, both CAR and mIL15-CAR T cells demonstrated specific lysis of $CD19^{+}$ tumor targets across all effector to target ratios with minimal lysis of $CD19^{neg}$ tumor targets (**Fig. 12A**). Another measure of T cell responsiveness to TAA was with the assessment of IFNγ production after six hours of co-culture with $CD19^{+}$ and $CD19^{neg}$ tumor targets, and no target and lymphocyte activating cocktail (LAC) served as baseline and maximal activation references, respectively. Both T cell groups had similar levels of IFNγ production in response to $CD19^{+}$ targets and almost no background stimulation from targets lacking CD19 (**Fig. 12B**). Both CAR and mIL15-CAR T cells maintained redirected specificity toward TAA. Thus, the genetically modified T cells that were generated are clinically relevant and suitable for adoptive transfer experiments.

Figure 11. Phenotype of *ex vivo* expanded mIL15-CAR T Cells. CAR and mIL15-CAR T cells were phenotyped after expansion at Stim 4 for expression of T cell surface markers associated with T cell phenotype and differentiation state. (A) The plot indicates frequencies of cells expressing CD3, CD4, CD8, CD45RA, CD45RO, IL-7Rα, CD62L, CCR7, CD27, and CD28 markers. Horizontal line indicates the mean value. **P* = 0.047, *n* > 4, paired t test. (B) Representative flow plot for one of four normal donors showing expression of CD62L and CCR7. (C) Representative histograms showing expression of CD27, CD28, and IL-7Rα (*shaded*) or isotype control (*black*), *n* = 8.

Figure 11. Phenotype of *ex vivo* expanded mIL15-CAR T Cells



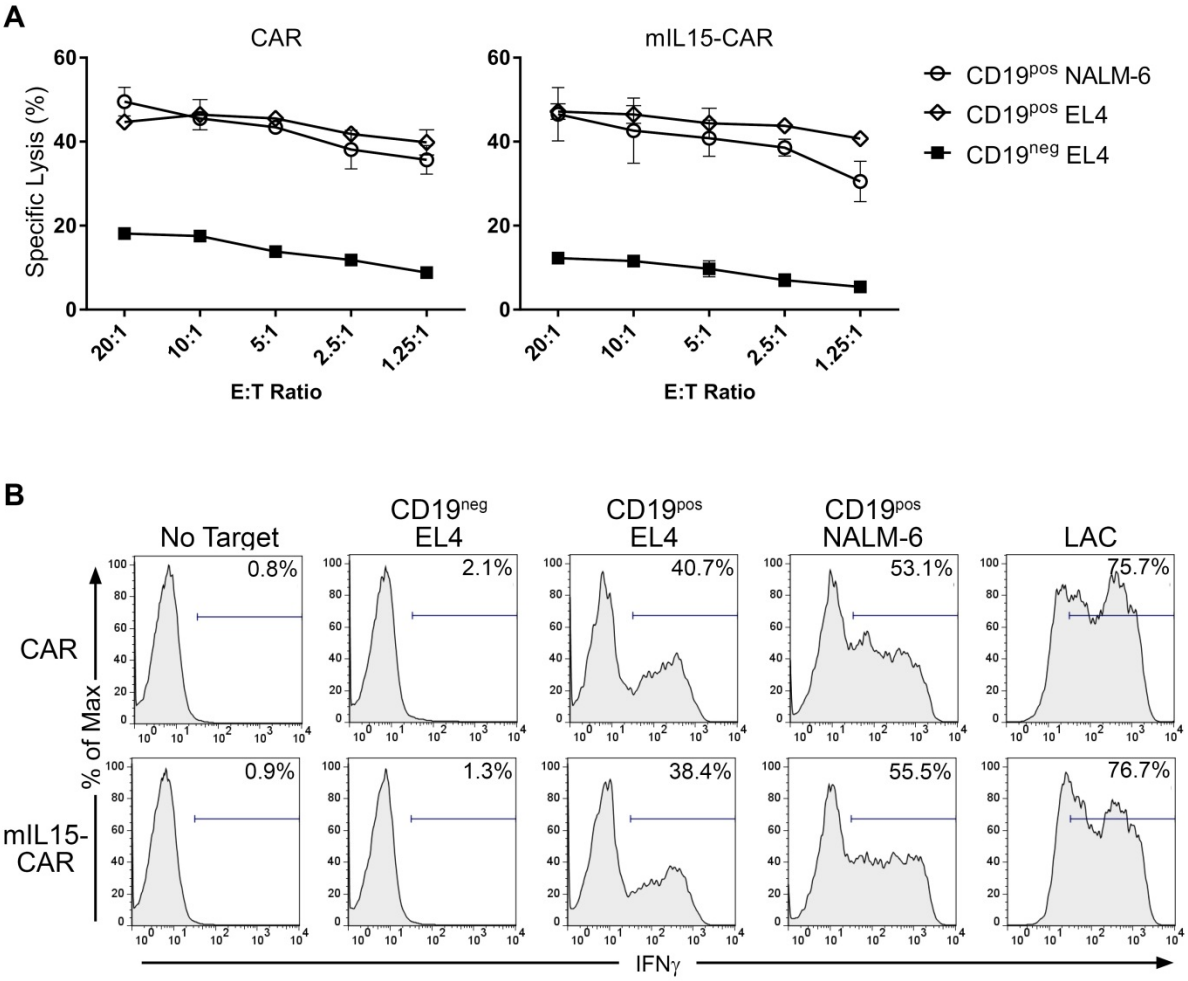


Figure 12. ***In vitro*** **effector function is not altered by mIL15 expression.** (A) CAR T cell (left panel) and mIL15-CAR T cell (right panel) specific lysis rates of $CD19^{+}$ or $CD19^{neg}$ targets from a four-hour chromium release assay. Data are represented as mean ± SD, *n* = 3. (B) IFNγ production by CAR and mIL15-CAR T cells after 6 hours of stimulation with $CD19^{+}$ ($tCD19^{+}$ EL4 and NALM-6) or $CD19^{neg}$ (EL4) targets, lymphocyte activating cocktail (LAC; positive control), or media alone (negative control). Histograms were gated on $CD3^{+}CAR^{+}$ cells. Representative flow plots for a normal donor are shown, *n* = 4.

2.3 Discussion

Attaining durable and robust CAR T cell persistence post-infusion continues to hamper ACT therapeutic potential. The approach undertaken in this study to address the issue of persistence was by co-modification of CAR T cells with a membrane-bound IL-15 fusion protein that would act to provide supplemental costimulatory signaling. This work aimed to generate pre-clinical data for the development of a Phase I clinical trial testing the therapeutic efficacy of mIL15-CAR T cells. In this section of the study, we aimed to generate mIL15-CAR T cells suitable for adoptive transfer that would be relevant for translation to the clinic. Using clinically compliant methods, mIL15 and CAR co-expressing T cells were generated and numerically expanded to numbers sufficient for clinical application. We showed that expression of mIL15 on CAR T cells resulted in sustained pSTAT5 costimulation and mimicked the transpresentation mechanism of IL-15. The data herein suggest that mIL15-CAR T cells warrant further study of their therapeutic potential *in vivo* and will be addressed in Chapter 3.

The foundation of the mIL15 design used in this study was based on a soluble hyperagonist IL-15/IL-15Rα-sushi fusion protein (RLI) that linked IL-15 to the sushi region of IL-15Rα (103). It has been identified that the sushi domain within IL-15Rα is responsible for the binding interactions between the cytokine and IL-15Rα (115). Mortier et al. (103) demonstrated that this configuration was functional in inducing biological effects (i.e., proliferation) and that the linker between the IL-15 and sushi domain did not interfere with binding interactions. In fact, it was found that the IL-15/IL-15Rα fusion protein containing the linker was more potent than complexed IL-15 and soluble IL-15Rα (103). This advantageous design was incorporated into mIL15 but extended to utilize the full-length native IL-15Rα structure to secure mIL15 to the cell surface. As it has been demonstrated that the cytoplasmic portion of IL-15Rα enables trans-endosomal recycling of IL-15 back to the cell surface (70), we chose to retain the cytoplasmic domain for the potential advantage it may offer in maintaining mIL15 protein expression.

The configuration of mIL15 demonstrated sustained pSTAT5 levels in mIL15-CAR T cells in cytokine unsupplemented conditions that abrogated pSTAT5 signal in conventional CAR T cells (**Fig. 10**). This indicates that mIL15-CAR T cells are exogenous cytokine independent and induces signaling of the IL-2Rβ/γC receptor complex. Since STAT5 signaling has been shown to be the primary pathway activated by IL-15R complex signaling

(116), this study only assessed pSTAT5 as an indicator of signal transduction activity but by no means suggests that it is the only signaling molecule induced by mIL15-induced signaling. It cannot be excluded that other STATs and other pathways, including MAPK and Akt, are activated and contribute to the biological responses of the modified T cells (27, 108, 117, 118). Rowley et al. (104), assessed cytokine signaling in murine $CD8^+$ T cells induced by a transpresented membrane-bound IL-15/IL-15Rα similar to our construct but lacking the cytoplasmic region. In addition to elevated levels of pSTAT5, pSTAT3 was moderately elevated and pSTAT1 was slightly elevated. Alterations in pAkt and pERK 1/2 were not observed. However, relative to the positive control (50ng/ml IL-15), pSTAT5 induction appeared to be quite weak (104) indicating that cytokine receptor activation may have been too low to foster signal transduction across multiple pathways. The study used CD8 cells isolated from naïve mice which may explain the low level of cellular activation, as naïve T cells express low levels of IL-2Rβ (52, 119). A chimeric cytokine receptor eliciting constitutive IL-2Rβ signaling expressed in genetically modified human T_{CM} showed demonstrable levels of both pSTAT5 and p-Shc (120) illustrating greater and more inclusive signal transduction in a T cell subset that has higher IL-2Rβ expression. Work investigating IL-2Rβ signaling at different intensities illustrated altered and divergent pathway utilization in response to cytokine receptor signal modulation (27). These studies collectively suggest that in our system: (i) the more moderate IL-2Rβ induction, as suggested by the pSTAT5 signaling, also likely stimulates other pathways to some level and (ii) that our cells would likely demonstrate varying responsiveness to mIL15 based on the modulation of IL-2Rβ levels that fluctuate with T-cell differentiation, with the potential of being insensitive to mIL15, such as with naïve T cells or in circumstances where IL-2Rβ becomes down-modulated.

It has now been established that transpresentation of IL-15 is the primary mechanism of action *in vivo* (94, 121) that sensitizes IL-15Rα^{neg}IL-15$\beta^+\gamma C^+$ cells to physiologic levels of IL-15 (103) and induces qualitatively different signaling than equivalent doses of soluble IL-15 (71). Since we are mimicking the membrane-bound configuration in our model, it is likely that for mIL15 to be most effective it should function in the dominant physiologic manner and engage in transpresentation. In this study, it was demonstrated that mIL15 could indeed be transpresented to mIL15^{neg} T cells and induce a moderate pSTAT5 signal **(Fig. 10C**). The capability to transpresent mIL15 may be advantageous *in vivo*. Studies have shown that antitumor activity was greater when IL-15 was transpresented to immune

cells within the tumor site than if monomeric IL-15 was secreted within the tumor microenvironment (98, 104). The caveat is that the activity of monomeric IL-15 is dependent on concentration and thus could potentially match the activity of transpresented IL-15 if a sufficiently high concentration could be secreted. However, should a higher concentration of soluble IL-15 be needed to achieve a therapeutic outcome, it is possible that the risk for toxicity may increase. It also cannot be excluded that mIL15 may signal to the modified T cells under additional mechanisms. The crystal structure of IL-15 bound to IL-15Rα along with biochemical data have suggested the possibility of IL-15 presentation in *cis* to the IL-15Rβ/γ_C complex on a cell (115). Subsequent *in vitro* analysis has indicated that *cis*-presentation can occur (122) and that signal transduction induced in *cis* versus in *trans* results in different dynamics of activation (123). The biological implications of these varying signaling mechanisms relative to immunotherapeutic strategies remain ambiguous.

Groups have generated CAR T cells that secrete IL-15 (91-93). Regarding expansion of modified T cells with aAPC stimulation in the absence of exogenous cytokines, our results (**Fig. 8A**) were concordant with Dotti et al. (93) in which expansion was achieved. However, results contrasted with Markley et al. (92) whose IL-15 transduced cell population declined after an initial phase of expansion and paralleled the 0.1 ng/ml soluble cytokine control. However, this difference may be due, in part, to the different methods of generating genetically modified T cells (viral vs *Sleeping Beauty* systems) and/or propagation conditions (APC type and supplemented cytokines). Unlike these two studies, we also observed severe overgrowth of NK cells at early time points during expansion (**Fig. 8B,C**) that prompted the use of IL-21 to promote CD8 T cell expansion. The reported synergy between IL-15 and IL-21 stimulation on the proliferation of murine CD8 T cells (107) was similarly observed in our mIL15-CAR T cell cultures, where the addition of IL-21 yielded greater than a log increase in expansion and a shift from a roughly equal mix of CD4 and CD8 T cells to greater than 80% CD8 T cells. This study showed that mIL15-modification did not alter redirected T cell specificity, which is in agreement with other studies assessing IL-15 and CAR co-modified T cells (91, 93, 124). Those studies went on to show *in vivo* persistence and antitumor activity that indicates the potential for *in vivo* performance of mIL15-CAR T cells, which we will address in Chapter 3.

Numerous studies have shown the importance of IL-15 in generating and supporting memory CD8 T cell subsets (85, 86), and IL-15 and IL-21 have demonstrated a synergistic effect on expansion of memory-phenotype CD8 T cells (107). Thus, it was anticipated that

a greater frequency of memory-associated markers (i.e., CCR7, CD62L, CD28, CD27, and IL-7Rα) would be observed in the mIL15-CAR T cell expansion product relative to that of CAR T cells. Moreover, it has been shown that different cytokines and cytokine cocktails impact the phenotype of the expanded T cells (53, 125, 126). Therefore, it was somewhat of an unexpected finding that the *ex vivo* expansion phenotype of mIL15-CAR and CAR T cells was relatively unaltered (**Fig. 11**). Our observation is congruent with a study that expanded T_{EM-} and T_{CM}-derived $CD8^+$ clones that resulted in similar *in vitro* phenotype, but divergent *in vivo* fitness (50). One explanation for such results may be resultant of culture methods. Studies that subjected modified T cells to multiple rounds of antigen stimulation observed fewer differences between various cytokine treatments in the expanded T cell product (50, 93). Conversely, studies that observed differences due to cytokine treatment tended to limit antigen stimulation to one round (125, 126). An additional factor that likely contributes to this observation is that Akt undergoes strong stimulation from integrated signals generated by cytokine, TCR, and CD28 signaling, which are all triggered during an encounter with APCs. When the Akt pathway undergoes strong stimulation, activation of Akt results in translocation to the nucleus where it phosphorylates FOXO1 and inactivates its pro-memory transcription factor activities, thereby allowing for T cell differentiation to progress (25, 127). It has been demonstrated that despite the similar *in vitro* phenotype of expanded T cells derived from T_{EM} and T_{CM} subsets, their performance markedly differed upon transfer *in vivo* into primates. Only the T_{CM}-derived T cells persisted and some showed reversion to a memory phenotype (50). Our phenotyping was descriptive of cells that had recently undergone several rounds of T cell activating stimuli. It is likely that the phenotype alters once the activating stimuli that dominate the signaling pathways cease, and it is likely that intrinsic elements then play a role in subsequent phenotypic changes. This aspect will be further investigated in Chapter 4. The observations by Berger et al. (50), suggest that the mIL15-CAR T cells generated in this study retain potential for *in vivo* persistence and this will be assessed in Chapter 3. In sum, the data presented in this chapter show the generation of clinically applicable mIL15-CAR T cells that possess exogenous cytokine-independent costimulation of STAT5.

CHAPTER 3

In Vivo Persistence and Antitumor Efficacy of mIL15-CAR T Cells

3.1 Introduction

ALL patients presenting with MRD can span a dynamic range of detectable residual leukemic cells. Initially, MRD was gauged by morphologic remission but now increasingly sensitive methods are being used to detect leukemic cells. The range extends from 0.00004% MRD by deep sequencing (128), to 0.001-0.01% by quantitative polymerase chain reaction and flow cytometry (129, 130), to 5% or higher by cytomorphology (131). The level of MRD is correlative with relapse, where molecular failure denotes a high-risk group that tends to be resistant to drugs and thus are candidates for SCT and ACT (132). Even patients with low-level MRD (0.001% < 0.01%) have clinical significance (133). Consequently, CAR T cells adoptively transferred to MRD patients are typically in a relatively TAA-poor environment. These modified T cells are CAR-addicted to receiving survival signaling and consequently may fail to expand and persist after adoptive transfer.

Clinical trials using CD19 CAR T cells have largely dealt with indolent or chronic B-cell malignancies rather than ALL and MRD, therefore the much of the existing CAR T cell persistence data is framed within that context. Brentjens et al. (78) have reported results from an ongoing phase I clinical trial where CAR T cells (2^{nd} generation CAR 19-28ζ) are being used to treat ALL patients with relapsed disease or MRD. Infusion of CAR T cells induced a MRD^{neg} status but it was also observed that higher T cell persistence was correlated with high tumor burden and there was eventual normal B cell recovery (78) suggesting that CAR T cells may not persist long enough to prevent relapse at a later time point and that CAR-dependent signaling is of limited help in this situation. However, long-term therapeutic evaluation wasn't possible as patients later underwent HSCT. Providing costimulation outside of the CAR by co-expressing CD80 and 4-1BBL was shown to improve persistence of infused 1^{st} generation CAR T cells (42). Yet similar to the 2^{nd} generation T cells, which receive CD28 costimulation via CAR activation, the addition of external CD80/4-1BB costimulation did not maintain durable T cell persistence and only aided T cells in a TAA-dependent manner. It is apparent that it is not a matter of just providing costimulation, but rather providing a signal that is context-dependent and suits the

conditions at a given time. IL-15's pleiotropic activities support T cells both during T cell encounter with antigens as an inflammatory cytokine, as well as in the absence of antigen as a homeostatic cytokine (86, 88). Hence, IL-15 may collaborate appropriately with T cells in the variable conditions associated with MRD.

In Chapter 2, we generated clinically relevant mIL15-CAR T cells that displayed sustained pSTAT5 suggesting that they may be more suitable than conventional CAR T cells in a low-TAA environment. To gather further preclinical data on mIL15-CAR T cells, we subsequently examined these cells *in vivo*. Their potential for *in vivo* performance is supported by studies demonstrating that IL-15 can enhance *in vivo* antitumor activity as well as support homeostasis of CD8 T_{CM}, which persist long-term without requiring contact with antigen (88, 134). We, therefore, **hypothesize** that mIL15-CAR T cells will possess enhanced *in vivo* antitumor activity and be capable of persisting *in vivo* irrespective of TAA.

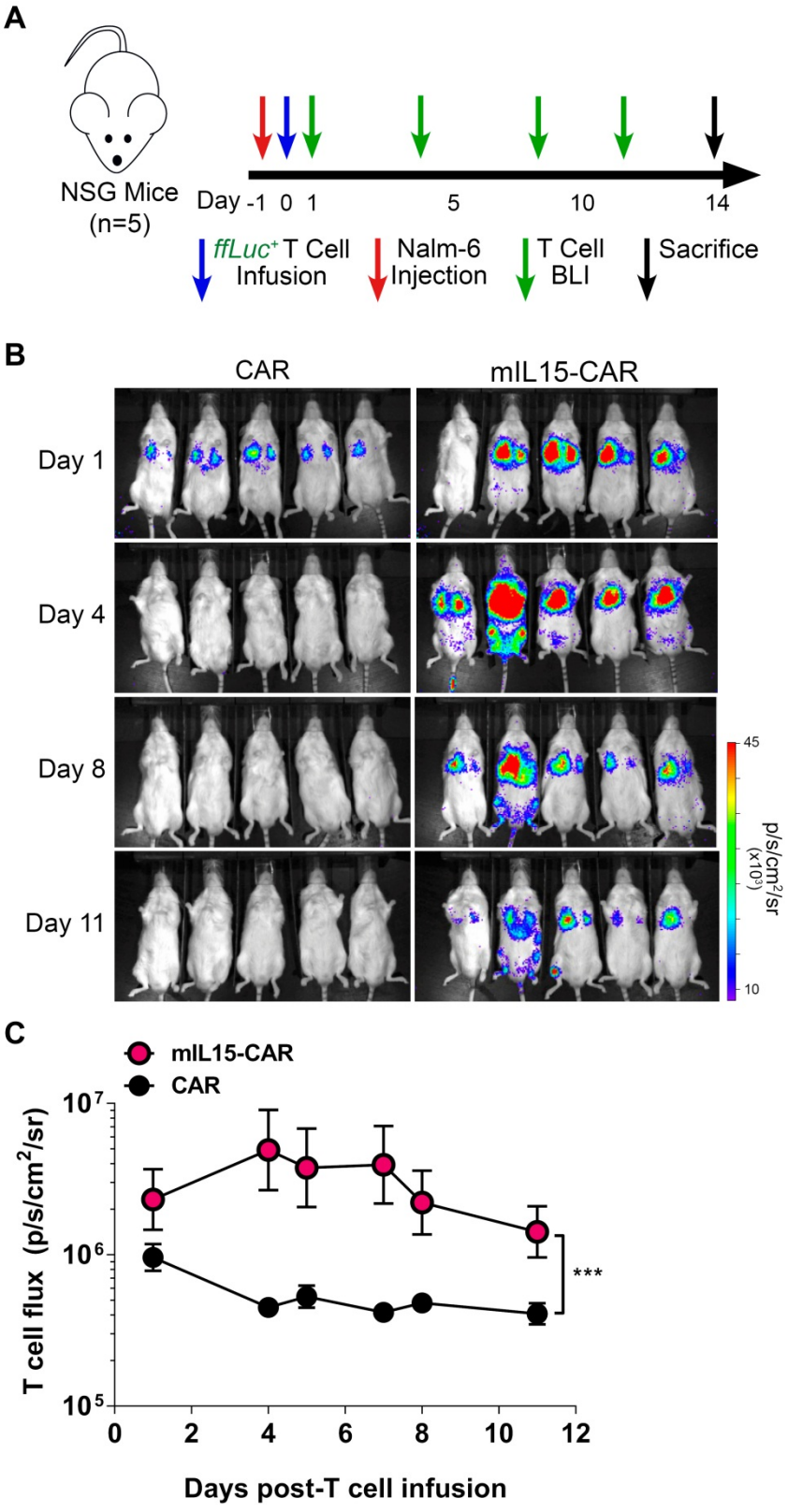
3.2 Results

3.2.1 Enhanced *In Vivo* Persistence of mIL15-CAR T cells in a High Tumor Burden Model

CARs have been designed to provide survival signaling upon engagement with TAA for improved *in vivo* persistence (41). In a high-dose disseminated leukemia model (1×10^5 NALM-6 tumor cells), it was previously shown that CAR T cells mediate significant antitumor activity (53). However, *in vivo* persistence of these cells in the presence of abundant tumor antigen remained unknown and had any assessments been made the weekly T cell infusions would have been confounding. Here we sought to (i) determine whether conventional CAR T cells persisted for any length of time in this high tumor burden model and (ii) whether additional costimulation from mIL15 would enhance CAR T cell persistence under prevalent TAA. CAR and mIL15-CAR T cells, co-modified with firefly luciferase (*ffLuc*), were adoptively transferred into NSG mice given a high-dose disseminated NALM-6 leukemia (1×10^5 cells i.v. injected; **Fig. 13A**). T cell persistence was longitudinally assessed by bioluminescent imaging (BLI) for T cell-associated *ffLuc* activity. Already on the first day after T cell infusion, CAR T cell persistence was observed to be lower than that of the mIL15-CAR T cells, with measured flux ranging $7.5 \times 10^5 – 8.9 \times 10^5$ p/s/cm^2/sr and $2.8 \times 10^5 – 4.0 \times 10^6$ p/s/cm^2/sr, respectively. The mIL15-CAR T cell-

Figure 13. Enhanced *in vivo* persistence of mIL15-CAR T cells when tumor antigen is prevalent. (A) Schematic of experiment. NSG mice were infused i.v. with 1 x 10^5 $CD19^+$ NALM-6 cells followed the next day by i.v. infusion of 2 x 10^7 CAR^+*ffLuc*$^+$ T cells with or without co-expression of mIL15. Infused T cell number was based on CAR^+ T cells. (B) Longitudinal BLI was performed to monitor T cell persistence from days 1 - 11. Images represent photon flux from T cell-derived *ffLuc* activity. (C) Longitudinal plotting of T cell flux. Data are represented as mean ± SD, $n = 5$. *$P = 0.0009$, unpaired t test.

Figure 13. Enhanced *in vivo* persistence of mIL15-CAR T cells when tumor antigen is prevalent



treated mice had BLI that was detected during the course of the study and remained with significantly higher flux than CAR T cell treated mice at day 11 ($P = 0.0009$; **Fig. 13B,C**). At 14 days, the mice were sacrificed and peripheral blood, spleen, and bone marrow were harvested to assess the presence of human CD3 cells. No human $CD3^+$ cells were detected in the blood or tissues of mice treated with CAR T cells. Mice receiving mIL15-CAR T cells showed human $CD3^+$ cells at frequencies of 9.1% - 52.8% in the blood, 0.8% - 11.0% in the spleen, and 0.4% – 1.6% in the bone marrow. Significant levels of chimerism were observed as compared to CAR T cell ($P < 0.05$, $P < 0.05$, $P < 0.001$, respectively) and tumor only-treated mice ($P < 0.05$, $P < 0.05$, $P < 0.001$, respectively; **Fig. 14A,B**). This type of disseminated tumor model using 1 x 10^5 Nalm-6 cells is relatively aggressive with mortality occurring in untreated mice around 21 days after tumor injection. Other studies using this tumor model treated mice with multiple CAR T cell infusions and while antitumor effects were observed, it was not a curative model (53). Hence, it was not unexpected that one infusion of mIL15-CAR T cells did not show significant antitumor activity. Together these data show that despite the presence of CAR-activating TAA, mIL15-CAR T cells but not CAR T cells persisted.

3.2.2 *In Vivo* Persistence and Antitumor Efficacy of mIL15-CAR T Cells in a Low Tumor Burden Model

Assessment of mIL15-CAR T cell persistence was next performed in a preventative low-TAA tumor model to assess transferred T cell engraftment and antitumor activity. In such a scenario, adoptively transferred T cells may not immediately encounter CAR-stimulatory TAA to receive survival signaling; hence, persistence may not be sustained. It has been previously shown that adoptively transferred CAR T cells (expressing a second generation CAR) only demonstrated TAA-dependent persistence (41), which thus poses concern for infusing CAR T cells into a low or sequestered TAA environment. In this preventative tumor model, CAR-*ffLuc* and mIL15-CAR-*ffLuc* T cells were first engrafted in mice followed by i.v. dissemination of a low-dose of NALM-6 cells (1 x 10^4 cells; **Fig. 15A**). CAR T cell BLI was undetectable at day six and only mIL15-CAR T cells persisted until the day of sacrifice (**Fig. 15B,C**). Total body flux was determined and was defined by a region of interest (ROI) that included the body and head, but not the tail. Total body flux showed mIL15-CAR T cell levels stabilize after 19 days post-infusion and remained significant at day 35 ($P = 0.0027$; **Fig. 15C**). BLI images of mIL15-CAR T cells at days 30 and 35 showed localization of cells to the tibia and foot region. Aggregations of T cells in those areas could putatively be within

Figure 14. Tissue distribution of persisting mIL15-CAR T cells under high tumor burden. At day 14, tissues and blood were harvested and analyzed by flow cytometry for the presence of $CD3^+$ T cells in the three treatment cohorts. (A) Representative flow plots show CD3 frequencies in the blood, spleen, and bone marrow, $n = 5$. (B) Plotted CD3 frequencies in the blood, spleen, and bone marrow from (A). Data are represented as mean ± SD, $n = 5$. $^*P < 0.05$, $^{***}P < 0.001$, one-way ANOVA.

Figure 14. Tissue distribution of persisting mIL15-CAR T cells under high tumor burden

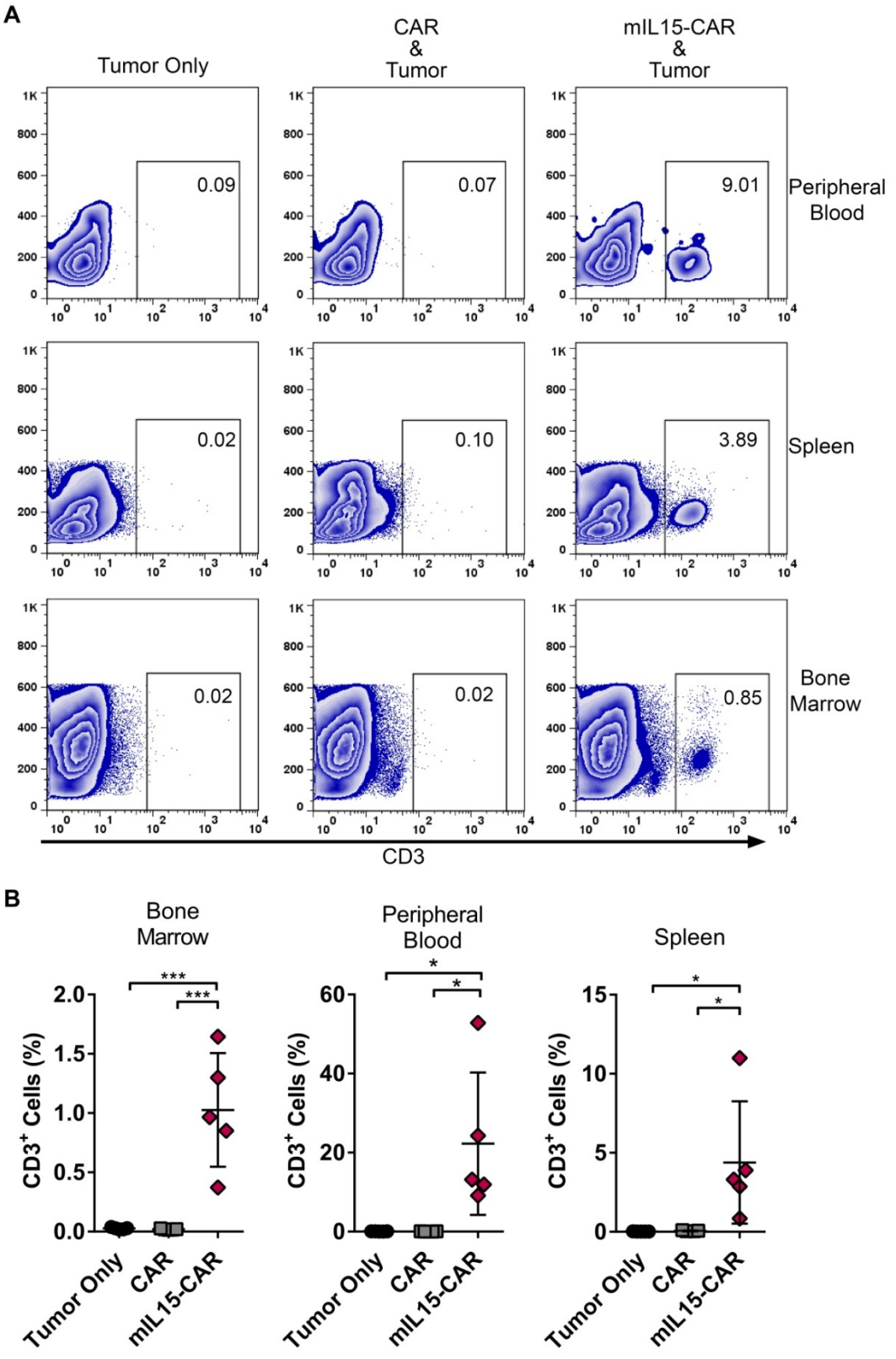
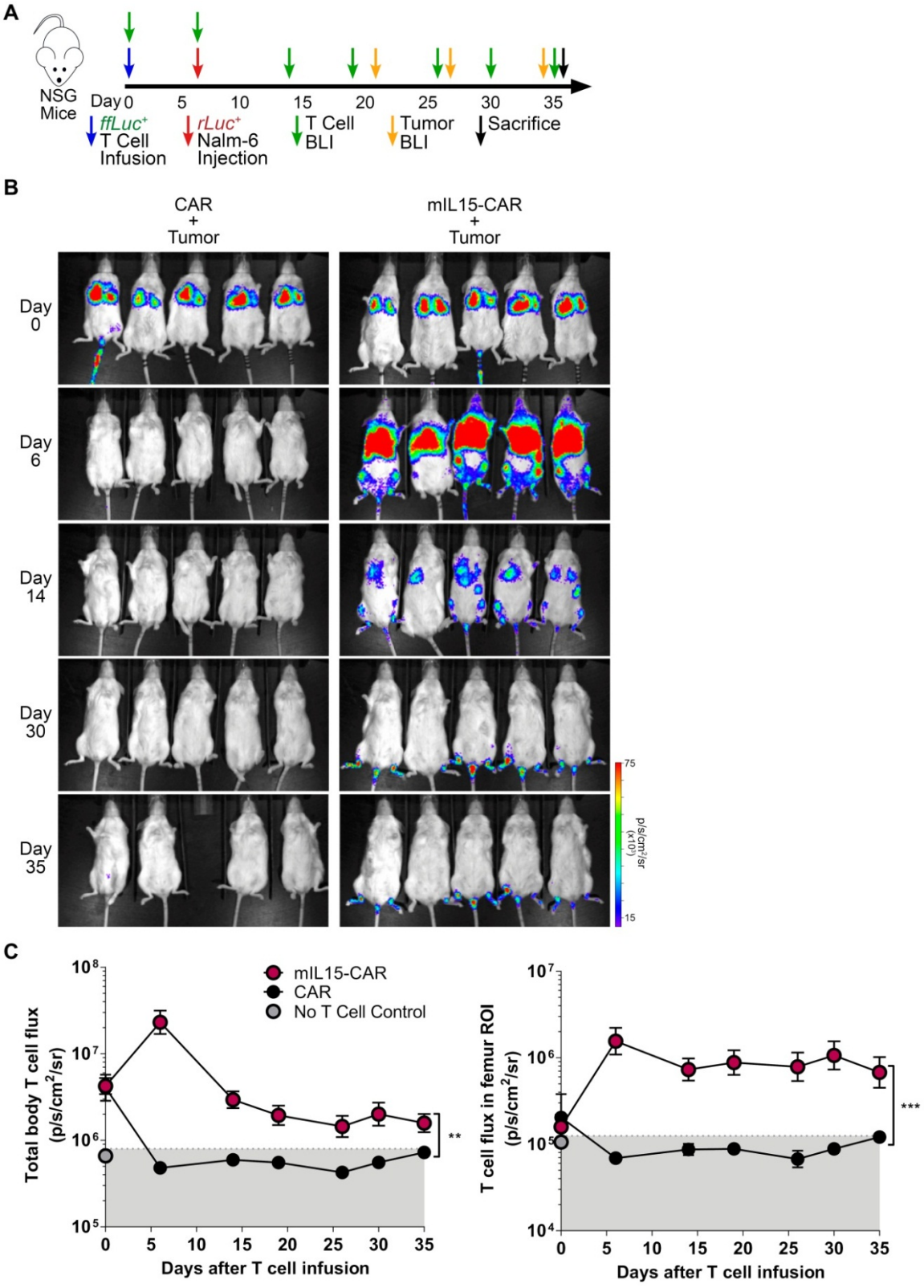


Figure 15. Enhanced *in vivo* persistence of mIL15-CAR T cells under low tumor burden. (A) Schematic of experiment. NSG mice were first infused i.v. with 2 x 10^7 CAR^+*ffLuc*$^+$ T cells with or without co-expression of mIL15. Infused T cell number was based on CAR^+ T cells. After six days of engraftment, 1 x 10^4 *rLuc*$^+$NALM-6 ($CD19^+$) cells were i.v. injected. (B) Longitudinal BLI was performed to monitor T cell persistence. Images represent photon flux from T cell-derived *ffLuc* activity. (C) Longitudinal plotting of T cell flux derived from ROIs encompassing the body (*left panel*) and the femurs (*right panel*). Background luminescence (*grey area*) was derived by imaging mice that received D-luciferin, but which did not receive *ffLuc*$^+$ T cells. Data are represented as mean ± SD, *n* = 4 – 5. ***P* = 0.0027, ****P* = 0.0007, unpaired t test.

Figure 15. Enhanced *in vivo* persistence of mIL15-CAR T cells under low tumor burden



the bone marrow and thus may also involve the femur region. Another ROI was created to hone in on each of the back legs and included the femur, tibia, and foot. The mIL15-CAR T cell flux from this region indicated stable engraftment in the bone marrow by the fifth day after T cell infusion and remained at a significant level at day 35 (P = 0.0007). The flux data do not indicate any trends of sustained uncontrolled exponential growth. Interestingly, while bone marrow flux remained persistent between days five thru 14, there was an observed sharp decline in mIL15-CAR T cells elsewhere in the body (**Fig. 15C**).

Tumor flux (NALM-6 *rLuc* activity) remained significantly lower in the mIL15-CAR mice compared to CAR-treated mice and tumor only mice demonstrating the antitumor activity of mIL15-CAR T cells (both $P < 0.0001$; **Fig. 16A,B**). Tumor eradication by mIL15-CAR T cells was corroborated by CD19 staining of peripheral blood, spleen, and bone marrow. No significant $CD19^+$ tumor burden (< 0.5%) was detected in the blood, spleen bone, or marrow ($P < 0.05$, $P < 0.0001$, $P < 0.01$, respectively; **Fig. 17A,B**). Notably, despite the tumor cells elimination from all of these locations, the mIL15-CAR T cells remained localized to the bone marrow (range: 0.1% - 2.8% of total cells), but their chimerism was at a significant frequency (P = 0.0050; **Fig. 17A,C**). No human $CD3^+$ cells were detected in the spleen or blood (**Fig. 17A**). The CD3 flow cytometry data corroborated the flux data that indicated a peripheral decline of mIL15-CAR T cells while the bone marrow environment remained stable.

Long-term evaluation of survival in this model showed enhanced therapeutic efficacy of mIL15-CAR T cell treatment resulting in 43% survival at 98 days as compared to 100% mortality in all other treatments by day 34 (**Fig. 18**). Survival of mice treated with mIL15-CAR T cells was significantly enhanced over mice treated with CAR-T cells (P = 0.045). One of the three surviving mice had a detectable population of human $CD3^+CD8^+$ cells at 0.220% in the bone marrow (data not shown). These data together indicate that mIL15-CAR T cells were capable of engrafting and mounting an antitumor response in a low TAA environment thereby making them more therapeutically effective in mice than conventional CAR T cells.

3.2.3 *In Vivo* Persistence of mIL15-CAR T Cells is CAR-independent

To assess if mIL15-CAR T cells can indeed persist long-term *in vivo* without the need for CAR-dependent survival signaling, modified T cells ($mIL15^{+/neg}CAR^+$*ffluc*$^+$) were infused

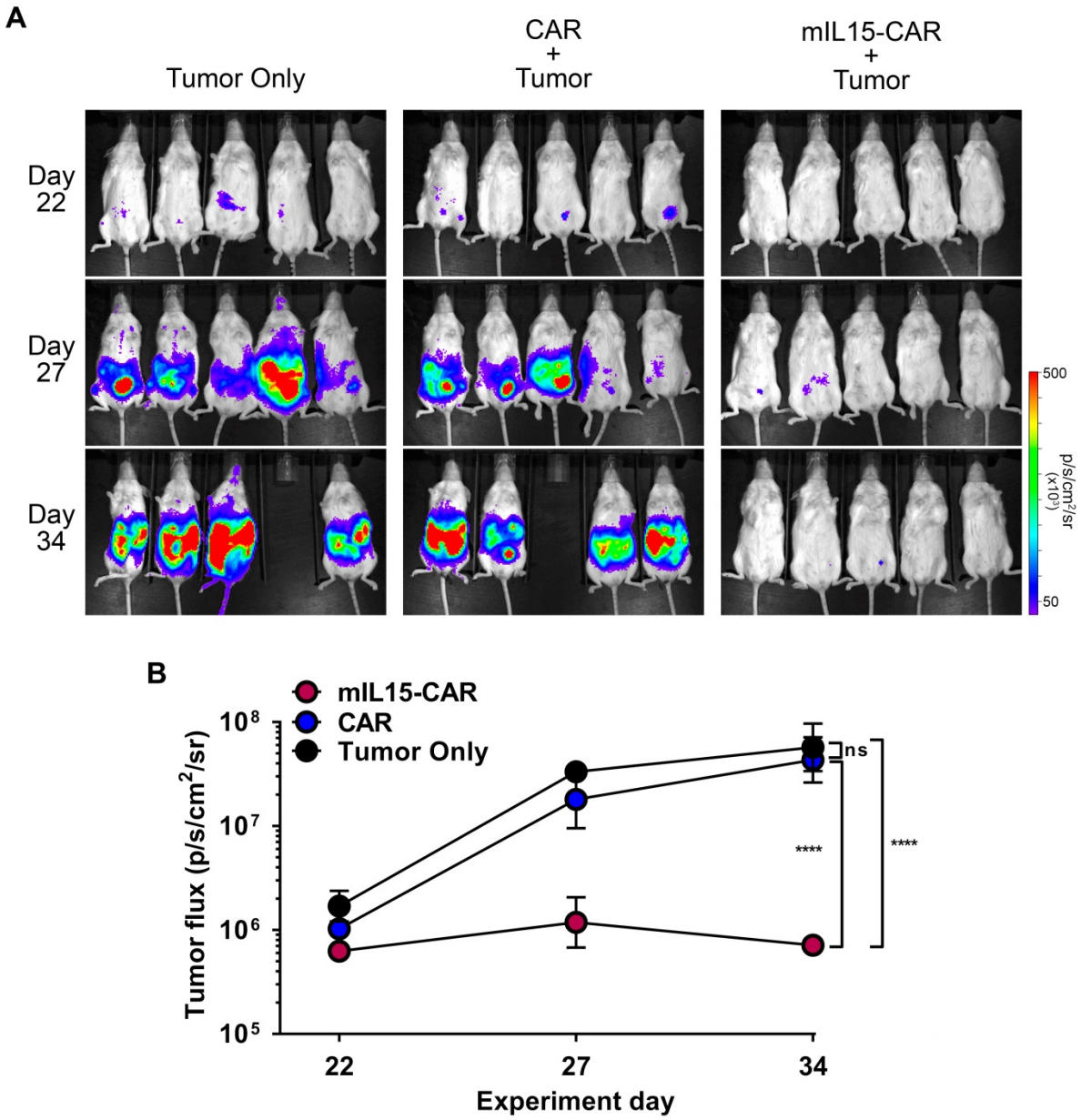
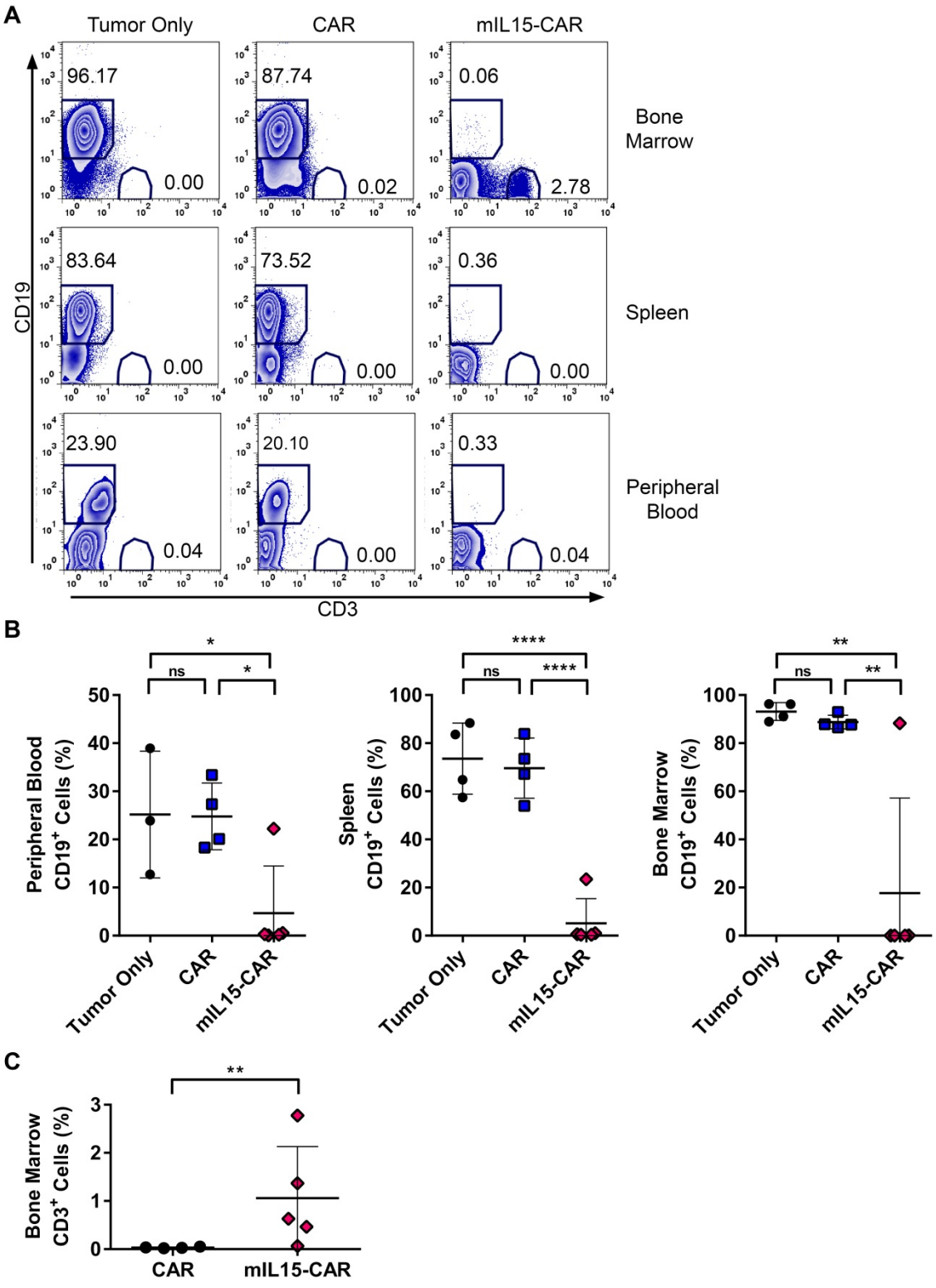


Figure 16. ***In vivo*** **anti-tumor activity of mIL15-CAR T cells.** (A) Longitudinal BLI was performed to monitor *rLuc*$^{+}$NALM-6 tumor cell persistence. Images represent photon flux from NALM-6 cell-derived *rLuc* activity. (B) Longitudinal plotting of tumor flux. Data are represented as mean ± SD, *n* = *4*-5. ****P < 0.0001, one-way ANOVA.

Figure 17. mIL15-CAR T cells clear tumor and remain localized to the bone marrow after an anti-tumor response. At day 36, tissues and blood were harvested and analyzed by flow cytometry for the presence of $CD3^+$ T cells and $CD19^+$ tumor cells in the three treatment cohorts. (A) Representative flow plots show frequencies of CD3 and CD19 in the peripheral blood (*n* = *3*-5), spleen, and bone marrow, *n* = *4*-5. (B) Frequency of $CD19^+$ cells in the blood (*n* = *3*-5), spleen, and bone marrow, *n* = *4*-5. (B) Plotted $CD3^+$ cell frequencies in the bone marrow. Data are represented as mean ± SD, *n* = *4*-5. **P* < 0.05, ***P* < 0.01, *****P* < 0.0001, ns = not significant, one-way ANOVA (B), unpaired t test (C).

Figure 17. mIL15-CAR T cells clear tumor and remain localized to the bone marrow after an anti-tumor response



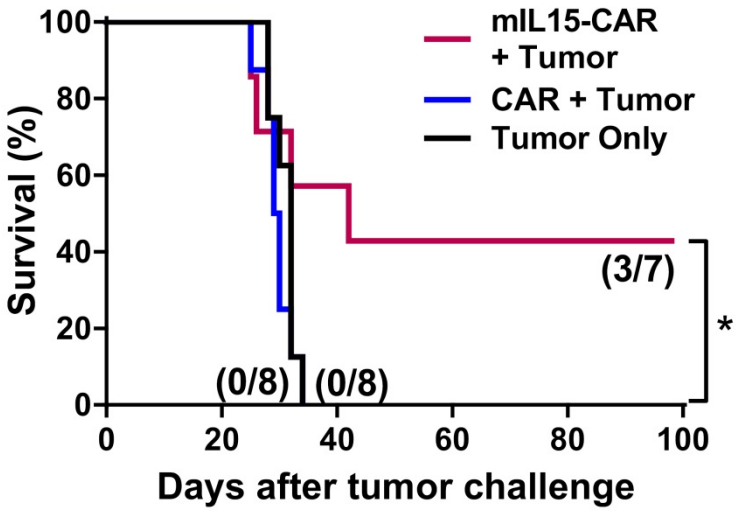


Figure 18. Efficacy of tumor immunotherapy with mIL15-CAR T cells. Using the low tumor model, long-term overall survival of mice was assessed to day 98. The experiment was carried out similarly to the previously described low tumor model. Mice were engrafted with mIL15-CAR T cells (*n* = 7), CAR T cells (*n* = 8), or no T cells (*n* = 8) followed by NALM-6 tumor challenge. Fractions in parentheses represent the proportion of mice surviving to day 98. **P* = 0.045 (mIL15-CAR versus CAR T cell treatment), log-rank (Mantel-Cox).

into mice with no tumor or exogenous cytokine support and monitored for 48 days (**Fig. 19A**). Akin to the previous results, CAR T cell persistence was not observed in this model after day zero, whereas mIL15-CAR T cells exhibited sustained persistence throughout the duration of the experiment (**Fig. 19B**). Both total body and femur flux of mIL15-CAR T cells at day 47 were at levels significantly above the baseline values of CAR T cells (P = 0.0128 and P = 0.002, respectively; **Fig. 19C**), but were not indicative of any trends of sustained and uncontrolled exponential growth. Blood and tissue analysis revealed that mIL15-CAR T cell engraftment was significant and primarily localized to the bone marrow and spleen (P = 0.0027 and P = 0.0081, respectively, **Fig. 20**). Although inconsistent chimerism was observed in the blood, it was associated with higher chimerism in the spleen and liver, as observed by BLI. Thus, it is suggested that mIL15 provides sufficient costimulation to sustain T cell persistence in a CAR-independent manner, which may make these T cells a more appropriate option for adoptive transfer in a low or sequestered TAA environment as may be the case with MRD.

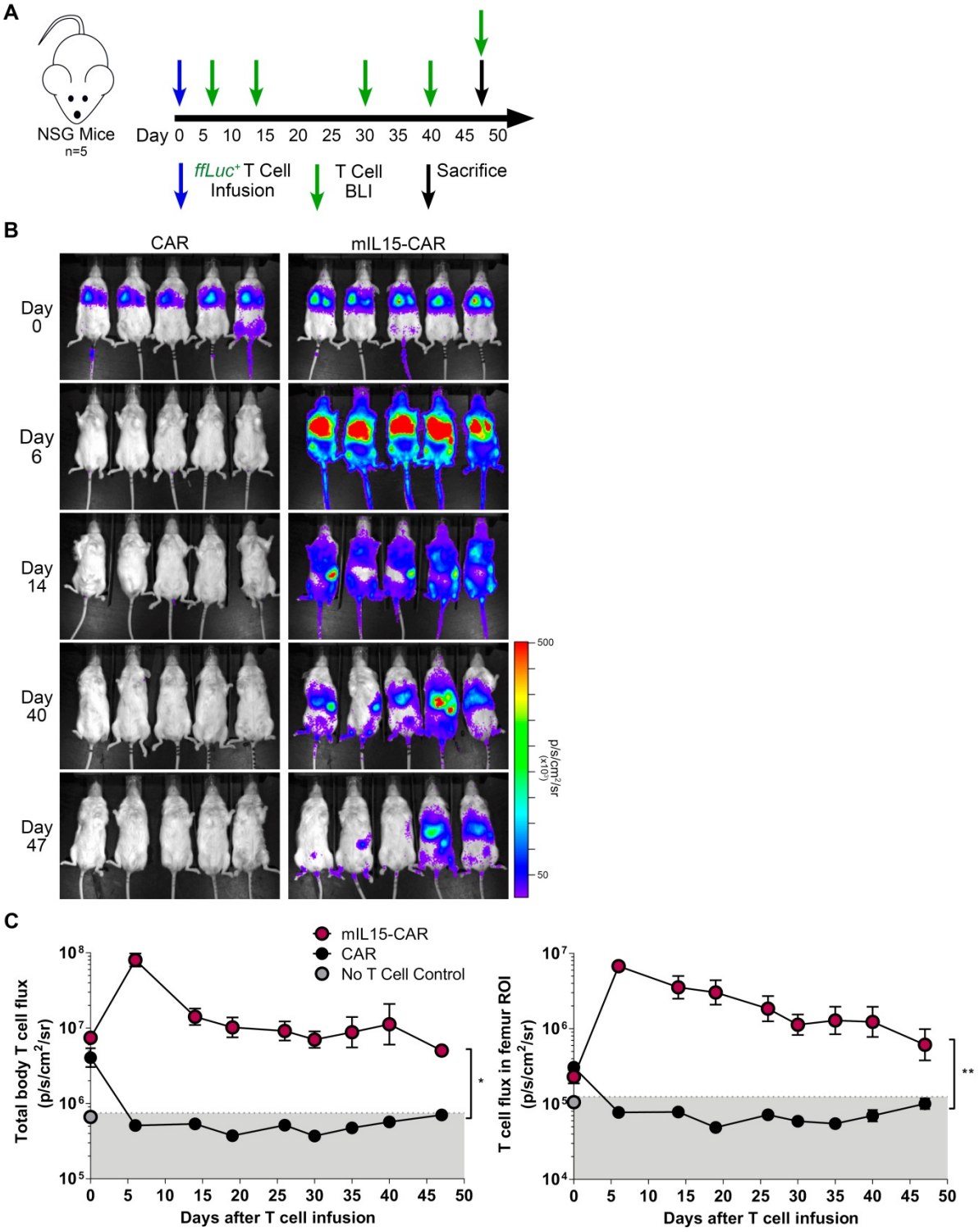
3.3 Discussion

Work in this chapter builds upon results from Chapter 2 providing the preclinical rationale for developing a phase I clinical trial using mIL15-CAR T cells to treat MRD. In contrast to conventional CAR T cells, mIL15-CAR T cells persisted in mice independent of the presence of TAA and eradicated $CD19^+$ leukemic cells in a low-dose disseminated tumor model indicating their suitability for translation to the clinic for the treatment of MRD.

CARs are designed with modular costimulatory endodomains, which in our model includes CD3ζ and CD28 signaling, to supply signals one and two for T cell activation (41). We observed that in the presence of abundant tumor, mIL15-CAR T cells showed enhanced persistence (**Fig. 13,14**) over the conventional CAR T cells, suggesting that IL-15 signaling provided a third additive costimulatory signal. The mechanism of this persistence was not investigated, but may be related to the prevention of AICD as IL-15 is reported to play a key role in resisting it (87). In addition to enhanced persistence under low tumor burden, mIL15-CAR T cells mediated significant antitumor activity (**Fig. 15-17**). These results are in concordance with other studies that evaluated CAR T cells modified to secrete monomeric IL-15 in leukemia models (92, 93). In those studies, CAR T cell persistence was assessed in reference to tumor exposure. We additionally examined

Figure 19. Long term *in vivo* engraftment of mIL15-CAR T cells in the absence of tumor. (A) Schematic of experiment. NSG mice were infused i.v. with 2 x 10^7 CAR^+*ffLuc*$^+$ T cells with or without co-expression of mIL15. Infused T cell number was based on CAR^+ T cells. (B) Longitudinal BLI was performed to monitor T cell persistence from days 0 - 47. Images represent photon flux from T cell-derived *ffLuc* activity. (C) Longitudinal plotting of T cell flux. Background luminescence (*gray shaded*) was defined by flux obtained from mice not receiving *ffLuc*$^+$ T cells. These mice were given D-luciferin and imaged along with the treatment mice. Data are represented as mean ± SD, n = 5, and representative of two similar experiments. *P = 0.0128, **P = 0.0021, unpaired t test.

Figure 19. Long term *in vivo* engraftment of mIL15-CAR T cells in the absence of tumor



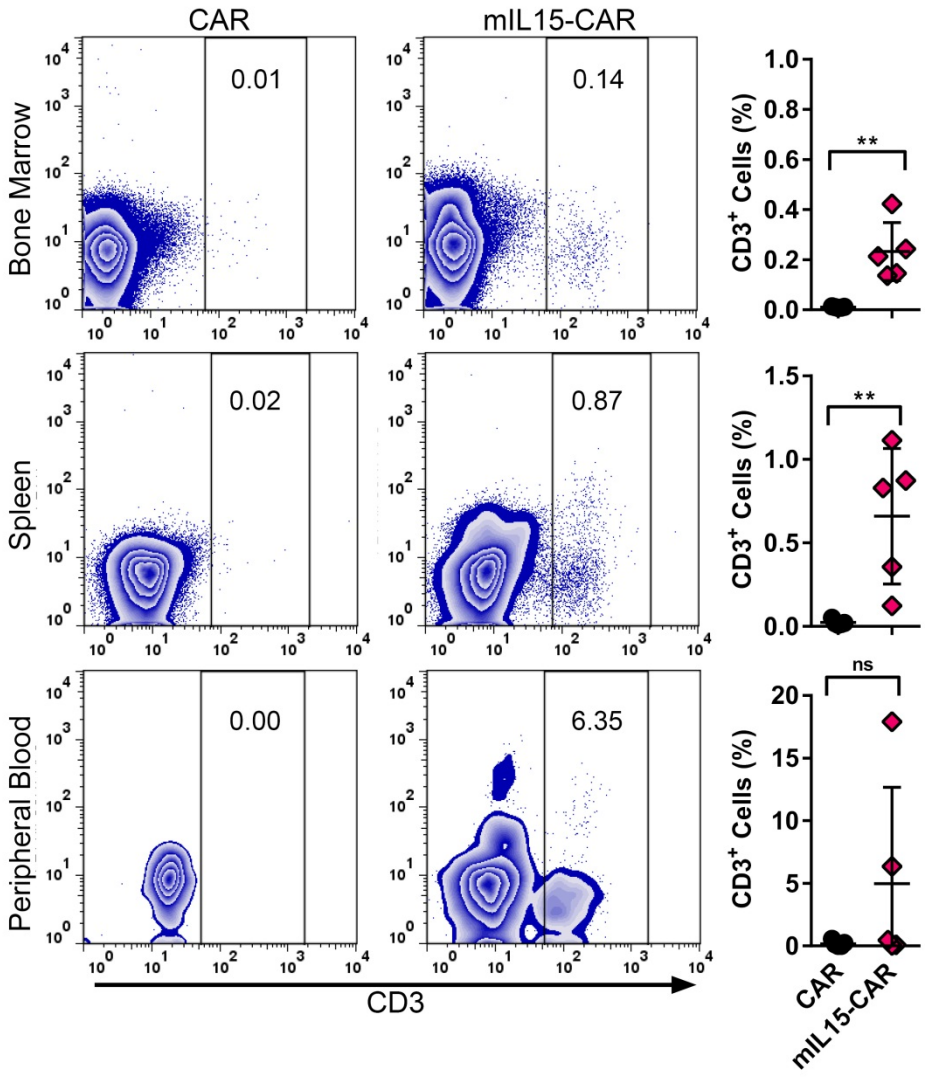


Figure 20. *In vivo* localization of mIL15-CAR T cells in the absence of tumor. At day 48, tissues and blood were harvested and analyzed by flow cytometry for the presence of $CD3^+$ T cells in mice treated with either mIL15-CAR or CAR T cells. Representative flow plots (*left panel*) show frequencies of CD3 in the peripheral blood, spleen, and bone marrow. Plotted frequencies of $CD3^+$ cells in the tissues and blood (*right panel*). Data are represented as mean ± SD, n = 5, and representative of two similar experiments. $^{**}P$ = 0.0027 (bone marrow), $^{**}P$ = 0.0081 (spleen), ns = not significant, unpaired t test.

persistence in the absence of TAA-dependent CAR signaling, where in the clinical setting of disease relapse or MRD, T cells may not encounter TAA for some time. We confirmed that mIL15-CAR T cells persisted independent of TAA (**Fig. 19,20**) and it is concordant with other studies where engraftment was achieved by T cells engineered to exploit the IL-2/15 cytokine signaling pathway by the expression of constitutively active STAT5 (135) or a chimeric cytokine receptor mediating constitutive CD122 signaling (120). Together, it is indicated that costimulation via IL-15Rβ/Cγ signaling is sufficient to support CAR T cell persistence allowing for continued antitumor activity and that persistence could be maintained in a low or no tumor environment.

Notably, we observed different levels and patterns of engraftment of mIL15-CAR T cells in this preventative low-dose tumor model (**Fig. 15,17**) compared to the no tumor engraftment model (**Fig. 19,20**). T cell abundance was inferred from the observed correlation of T cell levels with photon flux (42, 136), as well as our observations of photon flux and detected CAR T cell chimerism at sacrifice. We noted that mIL15-CAR T cell abundance during the antitumor response declined (**Fig. 13,15**) which contrasted other results of IL-15-secreting CAR T cells that showed expansion, or increased flux signal, in a tumor model (93). These observations highlight the issue of identifying what costimulation type and amount is sufficient in a tumor microenvironment to achieve optimal T cell function and expansion. Our *in vivo* model used Nalm-6 tumor cells that lack the CD80 and CD86 costimulatory molecules (137), whereas the other model (93) used Daudi and Raji cells which express both costimulatory ligands (137, 138). Moreover, it has been shown that enforcing expression of CD80 on Nalm-6 provided adequate costimulation to restore antitumor responses of CAR T cells (137). Nalm-6 tumor cells have been shown to suppress the up-regulation of costimulatory molecules on T cells affecting T cell biological responses (139). This may be a factor that contributed to (i) the extremely limited persistence of CAR T cells in the presence of TAA and (ii) the lack of expansion of mIL15-CAR T cells in response to TAA. These data cumulatively suggest that expression of mIL15 provides significant additive costimulation to enable persistence, but it is not fully compensatory under certain immunosuppressive conditions and emphasizes the importance of continued investigation of CAR designs that can provide fully competent T cell activation.

Another important observation was the differential chimerism that occurred in the preventative low-tumor mouse model as compared to tumor-free engraftment. Quite

strikingly, after the antitumor response, mIL15-CAR T cells in that model remained localized only to the bone marrow (**Fig. 15,17**). In contrast, mIL15-CAR T cells that engrafted in the absence of tumor were detected in the peripheral blood and spleen in addition to the bone marrow (**Fig. 19,20**). The observed retention of CAR T cells to the bone marrow was congruent with a syngeneic murine model of CAR T cells targeting B-ALL (140). In contrast, in a lymphoma model using the more costimulatory Raji tumor cell line in NSG mice, a definitive population of IL-15 secreting CAR T cells were observed in the spleens at over 106 days after tumor challenge (92). Perhaps this contrasting observation is due to the different time points of observation, as our results are reflective of T cell status at 36 days after tumor challenge. Assessments of murine CD8 T cells in the bone marrow suggest that this site is a niche for antigen-independent proliferation of memory CD8 T cells that display a more activated state than those from peripheral lymphoid organs (141, 142). Hence, it is plausible that in our model, repopulation of the spleen could occur at a later time. An additional aspect proposed to operate concomitantly is that repetitive antigen stimulation, and perhaps the suppressive nature of the Nalm-6, promoted a high degree of differentiation with subsequent loss of terminally differentiated cells. The observed bone marrow-resident population remaining after tumor challenge may not have undergone as extensive a differentiation, putatively due to being less differentiated at the time of infusion, having less contact with antigen, and/or having sufficient mIL15 costimulation. Nonetheless, this population remained to repopulate and thus resided in the bone marrow. It has been clearly illustrated that repetitive antigen stimulation induces differentiation that consequently affects function and longevity (17, 21, 143). Hombach et al (144) demonstrated that the transfer of less differentiated $CCR7^{+}$ CAR T cells results in a $CCR7^{neg}$ phenotype after exposure to antigen. The use of a 3^{rd} generation CAR with CD3ζ, CD28, and OX40 signaling supported the survival and effector function of these responding $CCR7^{neg}$ cells (144). Pairing mIL15 with this 3^{rd} generation CAR may assist in maintaining or even boosting CAR T cell levels during antitumor responses, hence yielding a larger resident population after the tumor is cleared. A readily translatable approach is to pair mIL15 with the 2^{nd} generation CAR that has CD3ζ and 4-1BB signaling capacity. This CAR is currently being used in clinical trials and has led to objective clinical responses (77, 145) and direct comparisons indicate that 4-1BB signaling may prove to be superior to CD28 signaling in CAR T cells both *in vitro* (146) and *in vivo* (H. Singh, unpublished data). Moreover, IL-15 has been shown to induce 4-1BB on T cells and 4-1BB/4-1BBL interactions sustain memory CD8 T cell survival independent of antigen (147). Cancer immunotherapy

with a targeted antibody fusion protein presenting IL-15 and 4-1BBL improved therapeutic efficacy over each as an individual treatment (148). The cooperative relationship between IL-15 and 4-1BB strongly suggests that combining mIL15 with the 4-1BB-signaling CAR holds significant potential for augmenting therapeutic response.

MRD detected by sensitive methods such as deep sequencing can detect extremely low levels of TAA in a patient (0.00004% MRD) (128) making delayed TAA encounter for CAR T cells a likely scenario. Our assessment of mIL15-CAR antitumor activity, therefor, utilized a preventative disseminated tumor model in which infused T cells would not immediately encounter TAA, and when tumor was present it was initially at a low level. Testing mIL15-CAR T cell antitumor activity could also have been diversified to include established subcutaneous tumor models using Raji or Daudi cell lines to assess antitumor activity with sequestered TAA. An immunocompetent syngeneic B-ALL murine model (140) could be used to assess the performance of mIL15-CAR T cells in treating MRD, which may be a more clinically relevant assessment of performance of adoptively transferred CAR T cells. This mouse model of B-ALL recapitulates disease pathology and mice may be conditioned with chemotherapy prior to adoptive T cell transfer for treating residual disease. Other elements to disease treatment with mIL15-CAR T cells could also be studied with this model, including immune reconstitution post-conditioning and adoptive transfer, and T cell memory subset composition throughout and antitumor response. The caveat with this approach is the requisite development of the murine CAR and mIL15 for use in this model.

The results described in this chapter showed long-term persistence of mIL15-CAR T cells and potent antitumor activity that enhanced therapeutic efficacy, as seen by significant long-term survival after tumor challenge. Together this indicates that mIL15-CAR T cells would be suitable for the treatment of MRD or sequestered TAA and supports the impetus to translate this approach to the clinic. However, it must be considered that further improvements may yet be made regarding enhancing the magnitude of T cells during and after tumor challenge in an immunosuppressive tumor model. Therefore, determining the optimal CAR signaling to pair with mIL15 remains an important consideration.

CHAPTER 4

Characterization of Long-term Persisting mIL15-CAR T Cells

4.1 Introduction

Intrinsic and extrinsic factors coalesce to dictate the fate of T cells and shape the composition of heterogeneous populations of effector and memory CD8 T cells. TCR signaling, costimulatory ligands, and inflammatory cytokines are the extrinsic factors providing the three signals required for T-cell activation (149-151). Additionally, γ_C cytokines are integral in T cell proliferation, survival, and memory. Intrinsically, numerous transcription factors have roles in T cell differentiation, including T-box expressed in T cells (T-bet), EOMES, T-cell factor 1 (TCF-1), BLIMP-1, and BCL-6 (reviewed in reference (16)). The unique amalgam of signals during antigen encounter results in varying degrees of differentiation with some T cells intrinsically biased toward a long or short-term fate (25, 26, 152). T cell differentiation is accompanied by phenotypic and functional changes. Less differentiated cells express CD62L and CCR7, have longevity, and can produce IL-2; whereas, those features are lost and effector functions are gained as differentiation progresses (16, 17). These functional features of T cell differentiation have implications on the therapeutic efficacy of ACT. It has been demonstrated that less differentiated T cells possess higher therapeutic potential (50, 52, 153) and the immunotherapy field is evaluating the importance of preserving T cell memory potential. Little knowledge exists on the characteristics and intrinsic mechanisms of long-term persisting CAR T cells. Moreover, questions exist as to the state of cytokine-modified CAR T cells, being that they receive continual cytokine signaling. With IL-15's well-known effects on the homeostatic maintenance of T_{CM}, we therefore **hypothesize** that persisting mIL15-CAR T cells will possess attributes associated with T_{CM}. Moreover, intrinsic elements will be representative of their differentiation state.

4.2 Results

4.2.1 Modeling Long-term Persistence *In Vitro*

Observing the long-term persistence of mIL15-CAR T cells *in vivo*, we aimed to model this in an *in vitro* setting to enable characterization of the persisting T cells. Expanded (Stim 4) mIL15$^{+/neg}$CAR^{+} T cells were put into long-term culture without further antigen re-stimulation. CAR T cells received IL-15 complex or no cytokine supplementation while mIL15-CAR T cells received no exogenous cytokines and were maintained for 65 days (**Fig. 21A**). Significant persistence of mIL15-CAR T cells was observed in comparison to cytokine unsupplemented CAR T cells, which were rapidly lost ($P < 0.0001$; **Fig. 21B**). By day 65 mIL15-CAR T cells appeared to show a gradual decline from their initial numbers ($P < 0.01$) whilst CAR T cells receiving IL-15 complex demonstrated an increased fold change in cell number resulting in significantly differing levels of persistence between the two groups ($P < 0.001$; **Fig. 21B**). Unlike the mIL15-CAR T cells, it appeared that soluble cytokine supplementation enabled CAR T cells to sustain growth within the first 15 days ($P < 0.05$), after which both groups did not demonstrate significant changes in cell number (**Fig. 21B**). CAR T cells supplemented with IL-2 paralleled the response of CAR T cells supplemented with IL-15 complex, suggesting that *in* the *vitro* T cell maintenance was not specific to IL-15 or mIL15 (data not shown). Moreover, the observed soluble cytokine-induced T cell growth may have been due to stronger cytokine signal induction from the dosing used, as pSTAT5 induction was stronger with the use of soluble cytokines (**Fig. 10** and unpublished observations). Further analyses of T cells persisting at the end of the withdrawal experiment refer to the cells as '**WD-T cells**' to denote this day 65 time point of the samples. Annexin V staining of cells remaining at the end of the experiment indicated that both WD-CAR and WD-mIL15-CAR T cells had similar viability (**Fig. 21C**). These data suggest that WD-mIL15-CAR T cells had sufficient costimulation to sustain T cell viability, but had inadequate stimulation for the growth that the exogenous cytokines induced and is a desirable characteristic in the context of homeostasis and safe long-term *in vivo* persistence.

4.2.2 CAR and mIL15 Expression and Functionality in WD-T Cells

While persistence is the primary aim of this study, surviving T cells would be of no clinical utility if they down-modulated expression of the CAR or mIL15, and/or if these

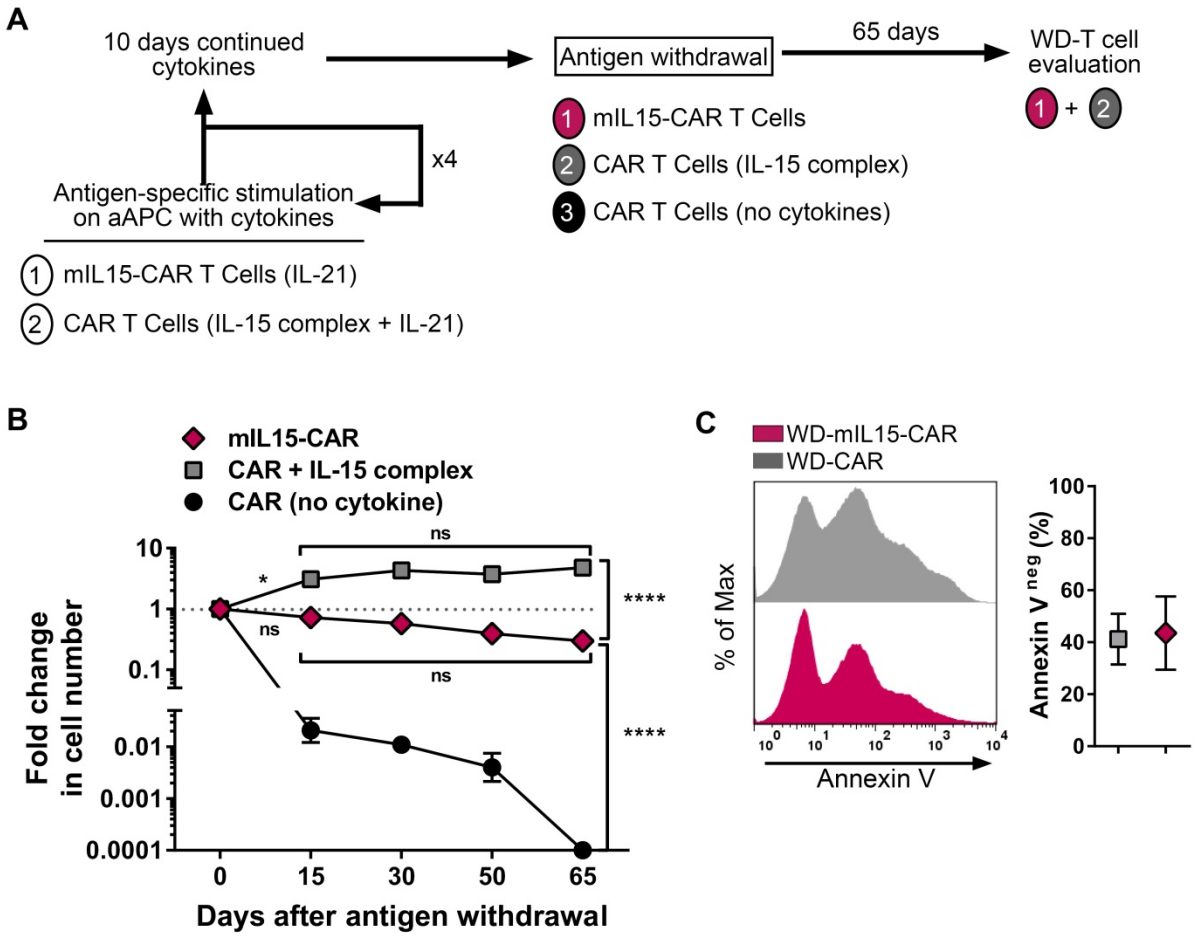


Figure 21. Modeling long-term persistence *in vitro*. (A) Schematic summarizing the withdrawal experiment for assessing long-term persistence of mIL15$^{+/-}$CAR^{+} T cells after withdrawal of antigen. T cells obtained for the withdrawal assay had been stimulated on aAPC four times and maintained for 10 days with respective cytokines used during expansion. During the 65-day exogenous cytokine withdrawal, the mIL15-CAR T cells received no cytokine supplementation while CAR T cell control treatments received either, IL-15 complex (5ng/ml) or no cytokines. (B) Fold change in number of T cells at 15, 30, 50, and 65 days during withdrawal conditions. Data represent mean ± SD, n = 3. *P < 0.05, ****P < 0.0001, ns = not significant, two-way RM ANOVA (Tukey's post-test). (C) Representative histogram showing Annexin V staining (*left panel*) and plotted live T cell (Annexin V^{neg}) frequencies (*right panel*).

molecules are non-functional. CAR and mIL15-CAR T cells entered the withdrawal assay expressing high levels of CAR (97% - 98% and 92% - 98%, respectively; Fig**. 22A**). The majority of WD-CAR and WD-mIL15-CAR T cells retained CAR expression (61 ± 20% and 82 ± 19%, respectively; mean ± SD; **Fig. 22B,C**). Unexpectedly, the WD-mIL15-CAR T cells showed a low surface expression of mIL15 (range: 13% - 81%). However, intracellular levels were detected at moderately high levels (range: 54% - 98%; **Fig. 22B,D**). Despite the majority of mIL15 being detected intracellularly, expression levels were sufficient to induce cytokine signaling that, compared to unsupplemented WD-CAR T cells, yielded elevated levels of pSTAT5 and Bcl-2, a pro-survival molecule downstream of STAT5 signaling (154-156) (**Fig. 23A**). Whether the intracellularly detected mIL15 participated in the induction of cytokine signaling is unknown. IL-15, bound to IL-15Rα, is known to persist without degradation in endosomes and recycle to the surface (71), thus allowing for the possibility that recycling kinetics may have altered. Notwithstanding, the pSTAT5 pathway was transducing cytokine signaling in WD-mIL15-CAR T cells. To be of benefit as a clinical application, CAR T cells must remain responsive to TAA, to which both WD-CAR (cytokine supplemented) and WD-mIL15-CAR T cells produced equivalent levels of IFNγ in response to $CD19^+$ targets ($P > 0.05$; **Fig. 23B**). Thus, despite the long-term absence of TAA, WD-mIL15-CAR T cells retained functional mIL15 and redirected CAR responses, which furthers their potential clinical utility.

4.2.3 Persistence of mIL15-CAR T Cells Does Not Involve Initial Molecular Programming

With deference to the crucial role that IL-15 plays in the homeostasis of $CD8^+$ T cell memory (85, 86), it was postulated and shown that mIL15 could support long-lived T cells in the absence of CAR or TCR signaling (**Fig. 19B,C; Fig. 21B**). Subsequent investigation sought to determine whether these long-lived T cells possessed traits associated with $CD8^+$ T cell memory. Some studies suggest that within the heterogeneous population of effector T cells there may be intrinsic programming predetermining cells to long or short-lived fates (157). Despite the observed phenotypic similarities between CAR T and mIL15-CAR T cells (**Fig. 11**), the possibility existed for cell intrinsic factors to play a role in the observed prolonged persistence of WD-mIL15-CAR T cells. To assess whether mIL15 conferred a survival advantage via molecular programming in the infusion product (Stim 4 expanded T cells), we compared CAR and mIL15-CAR T cells by digital gene expression analysis using a 479 gene lymphocyte-specific CodeSet (**Appendix 1**). Surprisingly, we found only four genes differentially expressed (2-fold cutoff, $P < 0.01$, FDR q value < 0.05; **Appendix 2**),

Figure 22. CAR and mIL15 expression on WD-T cells. (A) Representative flow plots showing CAR and mIL15 expression on T cells during the withdrawal assay at day 0 and (B) at day 65 (*top panel*), with FLAG staining detecting intracellular localization of mIL15 (*bottom panel*). (C) Frequencies of T cells staining positive for CAR. Horizontal bar represents the mean. (D) Frequencies of T cells staining positive for surface-detected and intracellularly-detected mIL15. Horizontal bar represents the mean. Data are representative of 3 donors.

Figure 22. CAR and mIL15 expression on WD-T cells

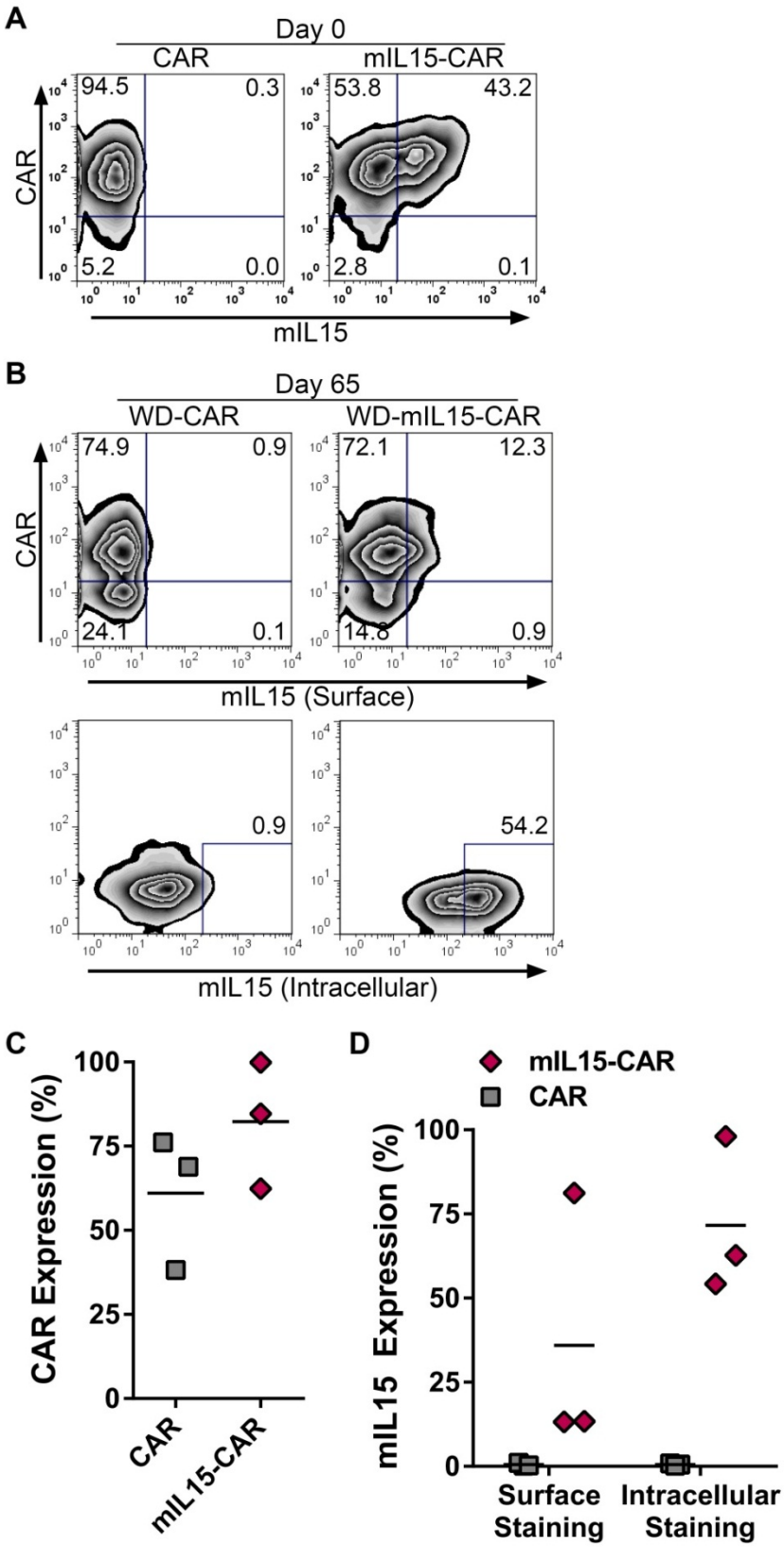
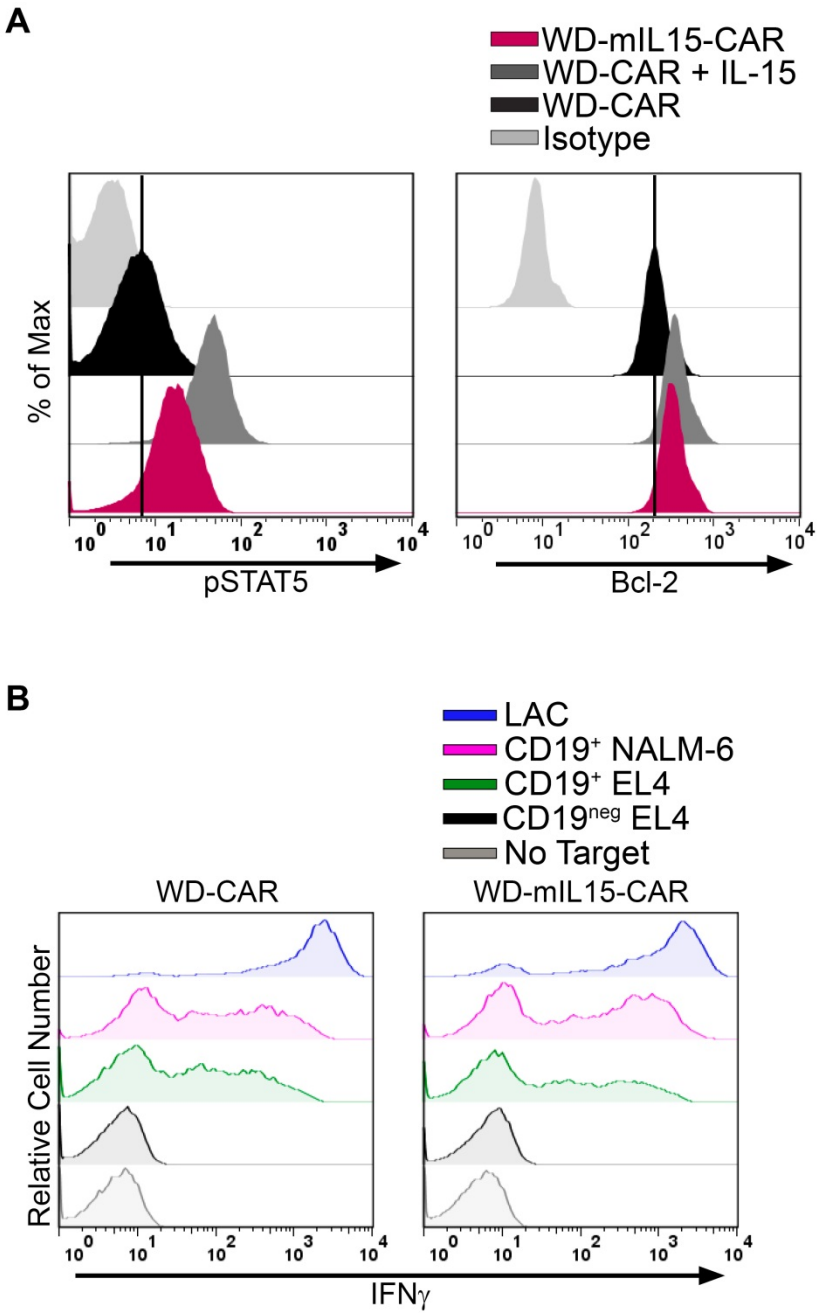


Figure 23. WD-mIL15-CAR T cells maintain pSTAT5 induction and antigen-specific reactivity. WD-CAR T cells supplemented with IL-15 complex and WD-mIL15-CAR T cells were assessed for functionality after antigen withdrawal. (A) Continued functionality of mIL15 was validated by phosflow of pSTAT5 and intracellular staining for Bcl-2 expression. For the pSTAT5 assay, samples were serum and cytokine starved for 12 hours. CAR T cells treated with soluble IL-15 (100ng/ml) 1-hour prior to fixation served as the positive control. For Bcl-2 analysis, two days prior to Bcl-2 staining, some CAR T cells were washed and resuspended in complete media without cytokine supplementation to serve as the baseline control. The representative histograms show the flourescence intensity of pSTAT5 (*left panel*) and Bcl-2 (*right panel*). (B) IFNγ production by WD-T cells stimulated for 6 hours with $CD19^+$ ($tCD19^+$ EL4 and NALM-6) or $CD19^{neg}$ (EL4) targets, LAC (positive control), or media alone (negative control). Histograms were gated on $CD3^+$ and CAR^+ cells. All data are representative of three donors.

Figure 23. WD-mIL15-CAR T cells maintain pSTAT5 induction and antigen-specific reactivity



with the difference in *IL7ra* expression corroborated by phenotyping (**Fig. 11A**). These results indicated no overt molecular survival advantage of mIL15-CAR T cells.

4.2.4. Molecular Profiling of WD-mIL15-CAR T Cells Describes a State of Low Differentiation

With the indistinguishable nature of CAR and mIL15-CAR T cells, subsequent investigation focused on identifying attributes of WD-mIL15-CAR T cells that are associated with long-lived T cells. Stark differences emerged when comparing long-term persisting WD-mIL15-CAR to Stim 4 mIL15-CAR T cells, where 98 genes were significantly differentially expressed (2-fold cutoff, $P < 0.01$, FDR q value < 0.05; **Fig. 24A,B**). Functional gene annotation was performed using DAVID Bioinformatics Resources (http://david.abcc.ncifcrf.gov) to cluster the significant genes into broad categories (158, 159). The most notable groups included the following: induction of apoptosis, regulation of cytokine production, homeostatic process, regulation of cell size, regulation of T cell differentiation, positive regulation of T cell activation, and Wnt signaling pathway (**Fig. 24C**). These functional clusters are relevant to processes affecting T cell differentiation status and suggest that aspects of differentiation may be a key factor involved in WD-mIL15-CAR T cell persistence.

Transcripts encoding key regulators of effector differentiation and related cytotoxic molecules showed diminished expression in WD-mIL15-CAR T cells: *Tbx21* (encoding T-BET), *Prdm1* (encoding BLIMP-1), *Il2ra* (encoding IL-2Rα), *Infg* (encoding IFNγ), *Prf1*, *Gnly*, *Gzmh*, *Gzmb*, *Cd11b*, and *Ctla4* (**Fig. 24A; Fig. 25A,B**). Inversely, a pro-memory transcriptional profile, indicative of a low level of differentiation, was represented by WD-mIL15-CAR T cells: *Tcf7* (encoding TCF-1), *Eomes*, *Bcl2*, *Bach2*, *Lef1*, *Bcl6*, *Foxp1*, *Ccr7* (encoding CCR7), and *Il7ra* (encoding IL-7Rα) (**Fig. 24B; Fig. 25A,B**) (16, 52, 160-163). The observed down-regulation of *Sell* (encoding CD62L) and *Cd28* (encoding CD28) by WD-mIL15-CAR T cells did not conform to the pro-memory transcriptional profile. IL-15 has been implicated in the generation of $CD28^{neg}$ CD8 T cells (164-166). Unexpectedly, *Foxo1* did not show a paralleled increased up-regulation with the other genes in the pro-memory profile, rather a non-significant decrease of 1.5-fold was observed in WD-mIL15-CAR T cells relative to mIL15-CAR T cells. This observation is rather perplexing as *Foxo1* is a transcription factor central to memory T cell differentiation in that it directly or indirectly regulates the expression of *Tcf7, Eomes, Sell, Ccr7,* and *Il7ra* (163, 167, 168). Instead,

Figure 24. Differential gene expression between mIL15-CAR T cells and long-term persisting WD-mIL15-CAR T cells. Multiplexed digital gene profiling was used to analyze gene expression for mIL15-CAR T cells obtained from cultures after the 4th aAPC stimulation cycle and WD-mIL15-CAR T cells persisting long-term (> 65 days) after antigen and exogenous cytokine withdrawal. Heat maps of significantly differentially expressed genes (2-fold differential expression cutoff, $P < 0.01$, and FDR q value < 0.05) that were (A) down-regulated and (B) up-regulated in WD-mIL15-CAR T cells relative to mIL15-CAR T cells, $n = 3$. (C) All significantly differentially expressed genes were functionally classified using DAVID Bioinformatics Resources. Select groups relevant to T cell persistence and differentiation status are displayed.

Figure 24. Differential gene expression between mIL15-CAR T cells and long-term persisting WD-mIL15-CAR T cells

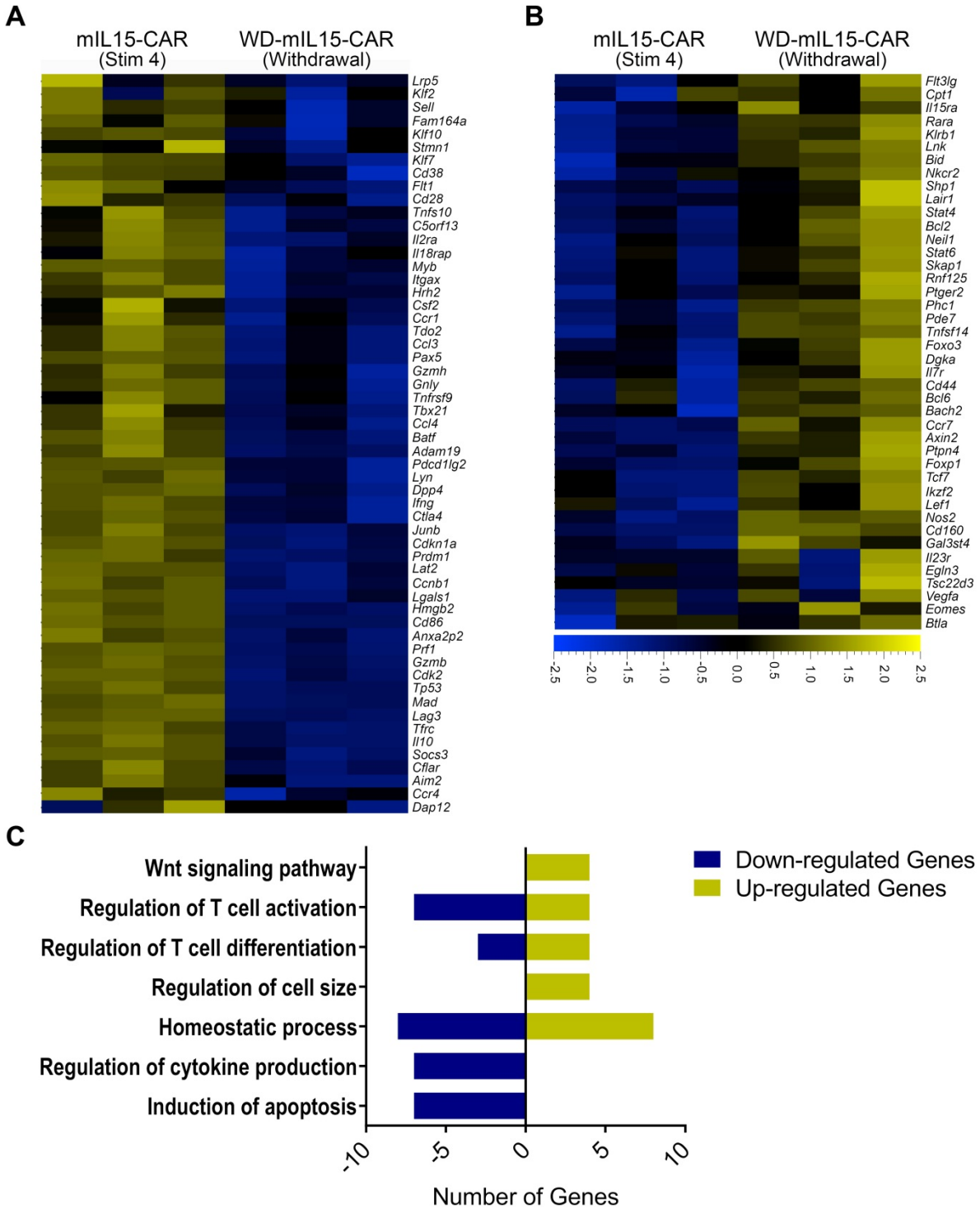
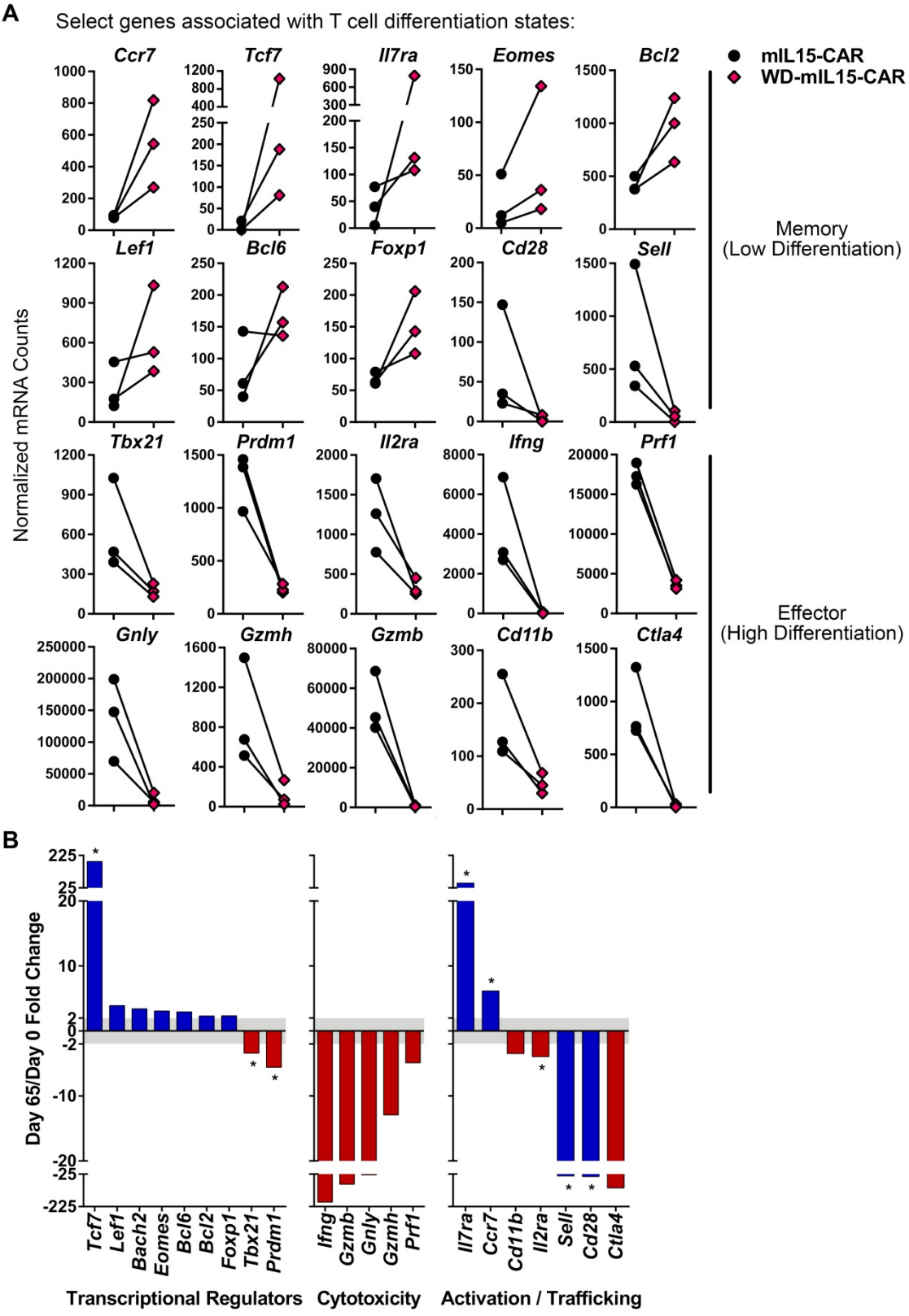


Figure 25. Molecular profiling of WD-mIL15-CAR T cells describes a state of low differentiation. Comparison of select significantly differentially expressed genes between expanded mIL15-CAR and persisting WD-mIL15-CAR T cells. The selected genes are associated with long-lived central memory or short-lived effector T cells, which are described as having low and high levels of differentiation, respectively. (A) Plots of normalized mRNA counts for selected genes from the multiplexed digital gene profiling. Lines connecting data points match donors. (B) Classification of the subset of genes in (A) by function. Bars represent the mean fold change in gene expression of WD-mIL15-CAR T cells relative to mIL15-CAR T cells. Asterisk indicates protein expression was verified by flow cytometry.

Figure 25. Molecular profiling of WD-mIL15-CAR T cells describes a state of low differentiation



Foxo3 was significantly up-regulated 2.1-fold (**Fig. 24B**), but its role in CD8 T cell differentiation remains ill-defined.

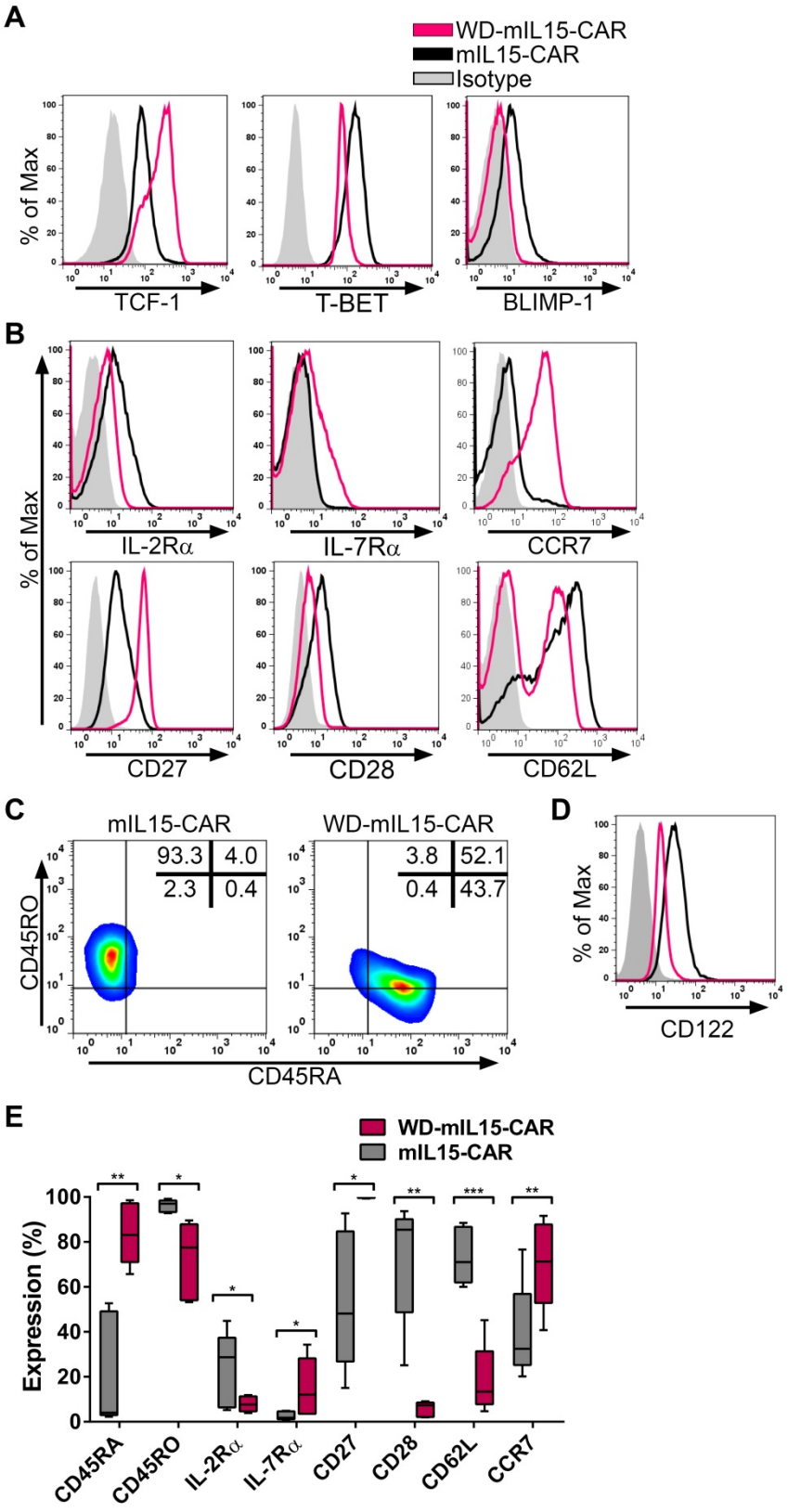
Some transcription factors have been found to function in pairs with graded reciprocal activity controlling effector and memory T cell differentiation. The ratio of *T-bet* and *Eomes* is one such pairing, with the ratio lowest in early memory T cell differentiation states (16, 26). We observed that mIL15-CAR and WD-mIL15-CAR T cells indeed had altered mRNA ratios of *T-bet* and *Eomes* (20:1–94:1 and 2:1–9:1, respectively), with WD-mIL15-CAR T cells representative of the low ratio associated with memory stages. Together these data suggest that in the absence of antigen WD-mIL15-CAR T cells exhibit an overall profile that is characteristic of less differentiated T cells.

4.2.5 WD-mIL15-CAR T Cells Possess Phenotypic and Functional Characteristics Associated with a Low Differentiation State

Protein expression of specific transcription factors and surface markers associated with T cell differentiation status corroborated the observed low differentiation molecular profile of WD-mIL15-CAR T cells. Memory-associated transcription factor TCF-1 (16) was increased in WD-mIL15-CAR T cells whereas effector-associated transcription factors, T-BET and BLIMP-1 (16), were concurrently decreased in these cells (**Fig. 26A**). Contrasting mIL15-CAR T cells, WD-mIL15-CAR T cells showed significantly greater frequencies of the memory-associated surface markers IL-7Rα (*P* = 0.0454), CD27 (*P* = 0.0148), and CCR7 (*P* = 0.0091) were observed (**Fig. 26B,E**). This protein-level expression pattern of transcription factors and surface markers corresponds with the molecular profile established in section 4.3.4 (**Fig. 25A,B**). WD-IL15-CAR T cell expression of IL-7Rα ranged 4% - 34%, CCR7 expression ranged 41% - 92%, and CD27 expression was greater than 99%. Although at a diminished expression level relative to mIL15-CAR T cells, $CD62L^+$ cells were not absent but present at an average frequency of 18% (**Fig. 26B,E**). WD-mIL15-CAR T cells re-expressed CD45RA to 76 ± 20%. While the majority of cells were $CD45RA^+CD45RO^+$ a substantial population of $CD45RA^+CD45RO^{neg}$ cells emerged within the culture (31 ± 17%, mean ± SD, *P* = 0.0233; **Fig. 26C,E**). CD122 (IL-2Rβ) expression is highly expressed on memory cells but has decreased expression on less differentiated T cell subsets, such as in naïve or memory stem cells (17, 119, 169). CD122 MFI on mIL15-CAR T cells (30 ± 8, mean ± SD) significantly differed from that found on WD-mIL15-CAR T cells (15 ± 3, mean ± SD; *P* = 0.0040; **Fig. 26D**).

Figure 26. WD-mIL15-CAR T cells possess phenotypic characteristics associated with less differentiated T cells. Validation of select genes found to be differentially expressed and phenotypic characterization of persisting WD-mIL15-CAR T cells compared to mIL15-CAR T cells. (A) Representative histograms of transcription factor expression for TCF-1, T-BET, and BLIMP-1. (B) Representative histograms of surface expressed molecules associated with T cell differentiation status. (C) Representative flow plots showing co-expression of CD45RA and CD45RO. Gate frequencies are displayed in the top right corner of the plots. (D) Representative histogram of CD122 expression. (E) Frequencies of respective markers from (B) and (C) represented as interleaved box and whiskers bars with the horizontal bar denoting the median. All data are representative of greater than five donors. *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, paired t test.

Figure 26. WD-mIL15-CAR T cells possess phenotypic characteristics associated with less differentiated T cells

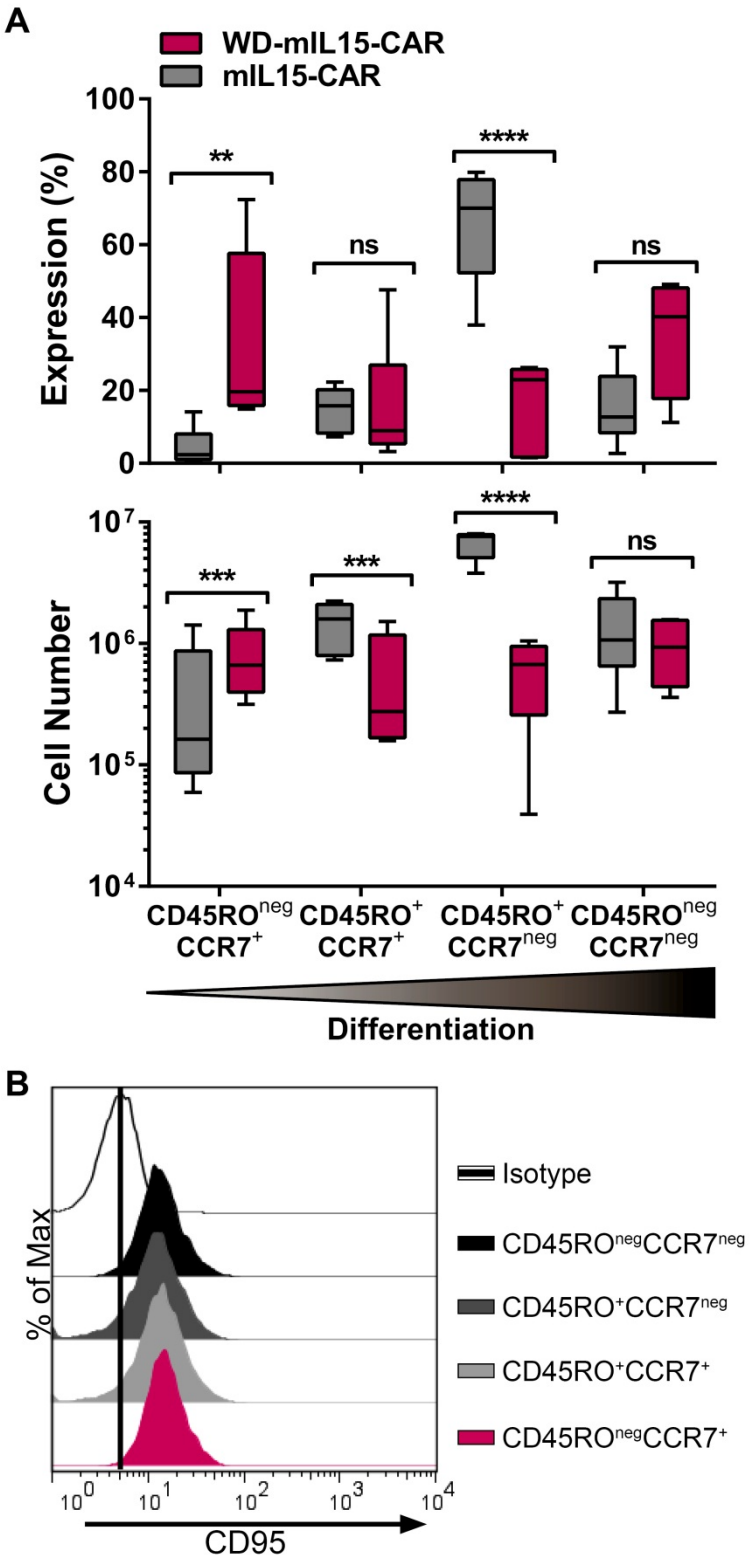


T cell subsets were assessed to further describe the changes in differentiation and memory potential. With the observed high co-expression of CD45RA and CD45RO, T cell subsets were defined by CD45RO and CCR7 expression (18, 170, 171). In increasing differentiation, the subsets are as follows: naïve or memory stem cell (T_N or T_{SCM}; $CD45RO^{neg}CCR7^{+}$), central memory (T_{CM}; $CD45RO^{+}CCR7^{+}$), effector memory (T_{EM}; $CD45RO^{neg}CCR7^{neg}$), and effector memory RA (T_{EMRA}; $CD45RO^{+}CCR7^{neg}$). *Ex vivo* expansion generates predominantly differentiated mIL15-CAR T cells with a $CD45RO^{+}CCR7^{-}$ effector memory phenotype ($P < 0.0001$ for comparisons of within mIL15-CAR T cell groups comparing memory subsets; **Fig. 27A**). After antigen withdrawal, the memory composition shifted to favor $CD45RO^{neg}CCR7^{+}$ WD-mIL15-CAR T cells ($P < 0.01$) and a sharp decline in the previously dominating $CD45RO^{neg}CCR7^{neg}$ subset was observed ($P < 0.0001$; **Fig. 27A**). The altered composition of CD45RO-CCR7 subsets at the end of withdrawal was resultant of a significant increase in $CD45RO^{neg}CCR7^{+}$ cell number (up to eight-fold) with concomitant decreases in cell number of $CD45RO^{+}CCR7^{+}$ and $CD45RO^{+}CCR7^{neg}$ subsets ($P < 0.001$, $P < 0.001$, and $P < 0.0001$, respectively). Surprisingly, $CD45RO^{neg}CCR7^{neg}$ T cells persisted long-term, albeit without increasing in number (**Fig. 27**). With the increase in $CD45RO^{neg}CCR7^{+}$ T cells, further examination was done to determine whether T cells in this subset were antigen-experienced as opposed to naïve. Further gating on the $CD45RO^{neg}CCR7^{+}$ subset of WD-mIL15-CAR T cells confirmed CD45RA expression (95% ± 4, mean ± SD), which may be expressed by either T_N or T_{SCM}. Assessing CD95 enabled differentiation between T_N or T_{SCM} subsets since T_N do not express CD95 (52). Additional staining showed that $CD45RO^{neg}CCR7^{+}$ T cells expressed CD95 similarly to the other antigen-experienced subsets (**Fig. 27B**). Together these data suggest that subsets supported by mIL15 during antigen withdrawal represent the least and most differentiated T cell subsets.

The net gain of this low differentiation subset corresponded with an acquired capacity for IL-2 production ($P < 0.0001$), a hallmark feature of memory cells (18). It was observed that merely 2-8% of mIL15-CAR produced IL-2 upon stimulation whereas 25-46% of WD-mIL15-CAR T cells produced IL-2 (**Fig. 28 A,B**). In sum, within the heterogeneous mix of WD-mIL15-CAR T cell subsets, T cells whose persistence was promoted or supported were $CD45RO^{neg}CCR7^{+}$ and $CD45RO^{neg}CCR7^{neg}$, respectively. Despite describing a bulk population, a molecular and phenotypic profile emerged showing that within the persisting subsets some T cells possessed traits associated with an early state of differentiation. The

Figure 27. Long term *in vitro* populations of WD-mIL15-CAR T cells become enriched with $CD45RA^{+}CCR7^{+}$ and $CD45RA^{+}CCR7^{neg}$ subsets. (A) Memory subset (based on CD45RO and CCR7) composition (*top panel*) and cell number (*bottom panel*) of mIL15-CAR T cells before and after withdrawal assay conditions. Data are plotted as interleaved box and whiskers with the horizontal bar denoting the median. Data is representative of greater than five donors. ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, ns = not significant, $n > 5$, two-way ANOVA (Bonferroni's post-test). (B) Representative histogram of one of four donors showing CD95 expression on WD-mIL15-CAR T cells amongst the memory subsets from (A). Histograms are gated on each of the corresponding subsets. The vertical bar in the plot denotes a reference line based on isotype staining.

Figure 27. Long term *in vitro* populations of WD-mIL15-CAR T cells become enriched with CD45RA^{+}CCR7^{+} and CD45RA^{+}CCR7^{neg} subsets



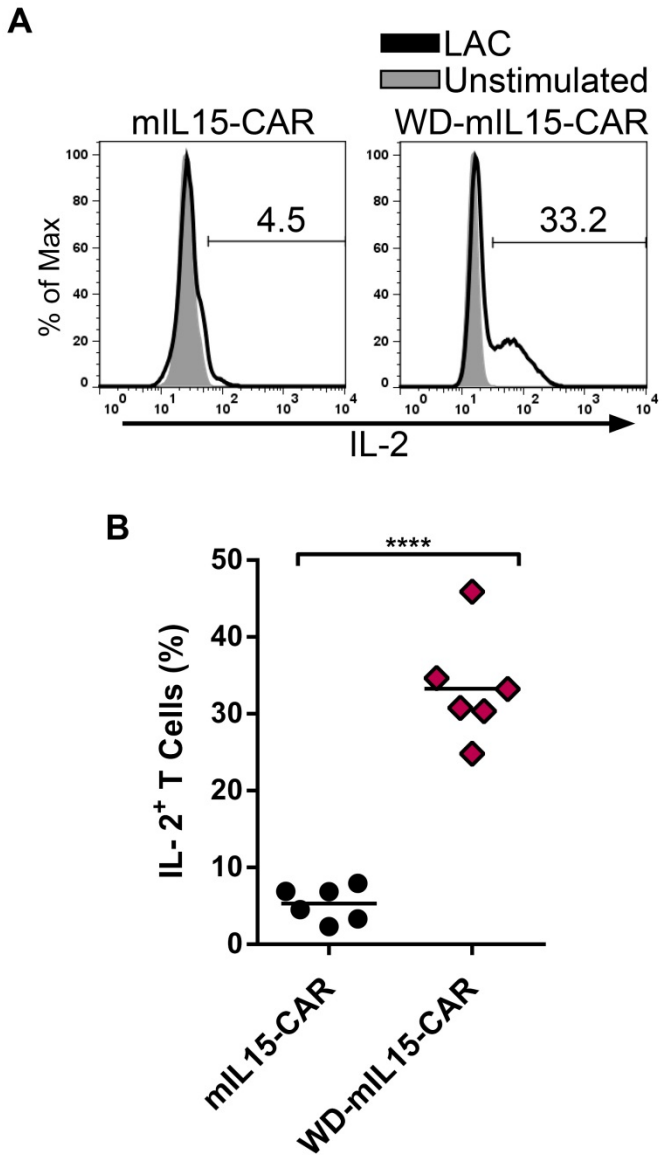


Figure 28. WD-mIL15-CAR T cells acquire the capability to produce IL-2. (A) Representative histograms of IL-2 intracellular staining of expanded mIL15-CAR T cells and persisting WD-mIL15-CAR T cells after lymphocyte activation cocktail (LAC) or no stimulation. (B) Frequency of LAC-stimulated T cells producing IL-2 from (A). ****P < 0.0001, n = 6, paired t test.

presence of such a phenotype for WD-mIL15-CAR T cells remaining after long-term antigen withdrawal strengthens its potential for clinical application.

4.2.6 mIL15 Supports CCR7$^+$ T Cell Subset Viability

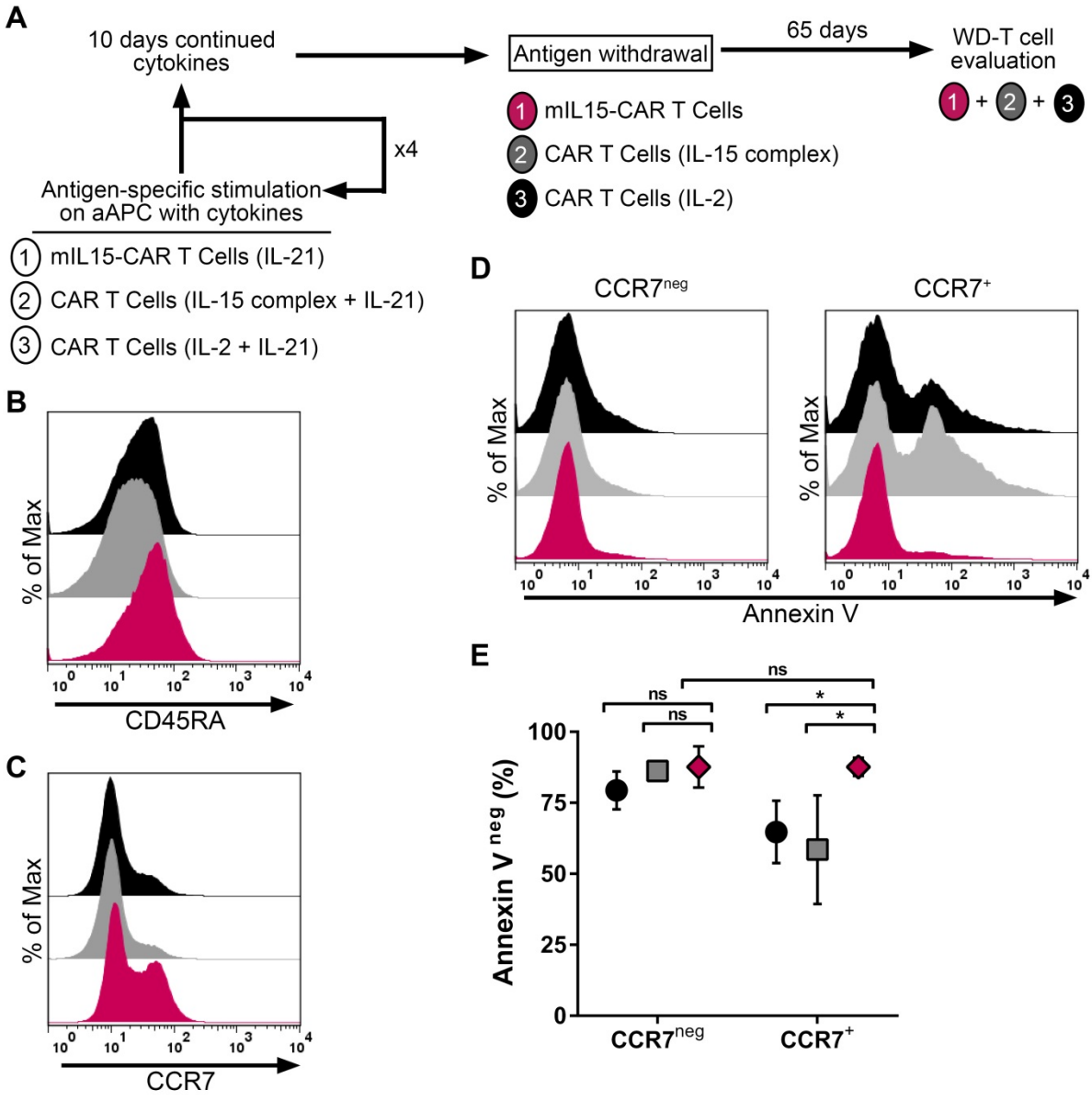
To determine whether the promotion of memory phenotype in our system was due specifically to mIL15, a 65-day withdrawal assay was conducted comparing WD-mIL15-CAR T cells to WD-CAR T cells cultured with soluble IL-2 or IL-15 complex (**Fig. 29A**). It was observed that CD45RA expression was elevated amongst the treatment groups (P > 0.05; **Fig. 27**). In contrast, CCR7 expression was 21% - 35% on WD-CAR T cells receiving IL-2, 17% - 31% on WD-CAR T cells receiving IL-15 complex, and 46% - 91% on WD-mIL15-CAR T cells (**Fig. 27C**). CCR7 expression was significantly enhanced in WD-mIL15-CAR T cells relative to CAR T cells supplemented with IL-2 or soluble IL-15 complex (both P < 0.05). Viability of CCR7^{neg} and CCR7$^+$ subsets was assessed by Annexin V staining. It was found that irrespective of the type of cytokine stimulation (IL-2, IL-15 complex, or mIL15), CCR7^{neg} T cells showed equal frequencies of live cells. In contrast, CCR7$^+$ T cells exposed to mIL15 had significantly higher viability than the CAR T cells receiving IL-2 or IL-15 complex (both P < 0.05; **Fig. 29D,E**). These data suggest that mIL15 is sufficient to support the CCR7$^+$ phenotype which thus contributes to maintaining the less differentiated CD45RO^{neg}CCR7$^+$ T cell subset.

4.2.7 mIL15-CAR T Cells Persisting *In Vivo* in the Absence of TAA Show the Emergence of an Early Differentiated CD45RO^{neg}CCR7$^+$ Memory Subset

The mIL15-CAR T cells were, once again, tested *in vivo* to further investigate memory subset maintenance and to determine whether the *in vitro* observations could be recapitulated. The mIL15-CAR T cells were adoptively transferred into NSG mice with no tumor or exogenous cytokine support and assessed after 50 days. T-cell expression of CD45RO and CCR7 was observed in a tissue-specific manner. T cells highly expressed CD45RO in the bone marrow, but CD45RO$^+$ T cells were at low frequencies in peripheral blood and spleen (**Fig. 30A**). Interestingly CCR7$^+$ T cell frequency was inversely associated with CD45RO expression where higher expression was observed in the blood and spleen (**Fig. 30A**). T cell differentiation, in the blood and spleen, recapitulated *in vitro* observations of the preferential persistence of CD45RO^{neg}CCR7$^+$ and CD45RO^{neg}CCR7^{neg} T cells (**Fig. 30B**). Strikingly, the bone marrow contained the highest proportion of CD45RO$^+$CCR7$^-$ T cells and nearly all of the T cells in the bone marrow were found to be

Figure 29. Tethered IL-15 supports survival of CCR7^{+} CAR-modified T cells. (A) Schematic summarizing the withdrawal experiment comparing IL-2, IL-15 complex, and mIL15 on CD45RA and CCR7 phenotype. During the 65-day antigen withdrawal, the mIL15-CAR T cells received no exogenous cytokine supplementation while CAR T cell control treatments received either IL-2 (50 U/ml) or IL-15 complex (5 ng/ml). T cells persisting at the end of the assay are referred to as WD-T cells. (B) A representative histogram of CD45RA expression and (C) CCR7 expression on WD-T cells. (D) Representative histograms showing Annexin V levels in CCR7^{neg} and CCR7^{+} WD-T cell subsets. Histograms were gated on the lymphocyte population to avoid non-specific CCR7 staining. (E) Graph plotting frequencies of live (Annexin V^{neg}) WD-T cell treatment cohorts in the CCR7^{neg} and CCR7^{+} subsets. Data are represented as mean ± SD. *P < 0.05, ns = not significant, two-way ANOVA (Tukey's post-test). All data are representative of three donors. Color legend: WD-CAR T cells supplemented with IL-2 (*black*), WD-CAR T cells supplemented with IL-15 complex (*grey*), and WD-mIL15-CAR T cells (*magenta*).

Figure 29. Tethered IL-15 supports survival of CCR7^{+} CAR-modified T cells



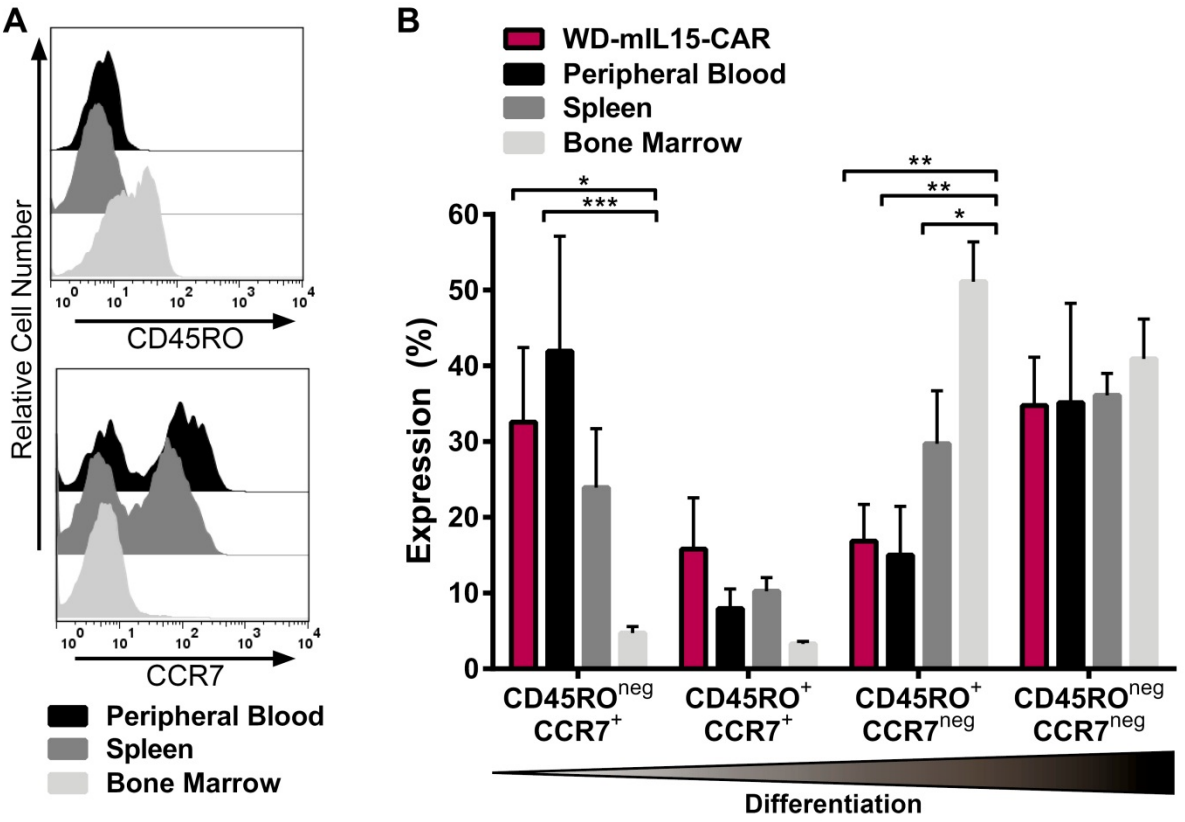


Figure 30. Long-term *in vivo* persisting mIL15-CAR T cells are maintained at diverse levels of differentiation with different distribution patterns. NSG mice were i.v. injected with 20 x 10^6 mIL15-CAR T cells. After 50 days analysis of peripheral blood, spleen, and bone marrow was performed to assess differentiation of T cells persisting long-term in the absence of CAR activation. (A) Representative histograms of CD45RO (*upper panel*) and CCR7 (*lower panel*) expression on *in vivo* mIL15-CAR T cells. (B) Memory subset composition of the *in vivo* T cells in reference to *in vitro* WD-mIL15-CAR T cells. Graphed data is represented as mean ± sem. **P* < 0.05, ***P* < 0.01, *** *P* < 0.001, two-way ANOVA (Tukey's post-test).

the more differentiated subsets (P < 0.0001 for $CD45RO^{+}CCR7^{neg}$ versus $CD45RO^{neg}CCR7^{+}$ or $CD45RO^{+}CCR7^{+}$; P < 0.001 for $CD45RO^{neg}CCR7^{neg}$ versus $CD45RO^{neg}CCR7^{+}$ or $CD45RO^{+}CCR7^{+}$; **Fig. 30B**). Similar to *in vitro* observations, T cells in the blood and organs expressed moderate levels of CAR and modest levels surface-detected mIL15 (**Fig. 31AB**). After *ex vivo* numeric expansion, T cells derived from the organs retained antigen-specific function by the production IFNγ in response to $CD19^{+}$ targets (**Fig. 31C**). Together these data suggest that while mIL15-CAR T cells can persist *in vivo* long-term as various memory subsets, they are also maintained as a substantial subset of early differentiated cells, which are thought to be a desirable population for ACT.

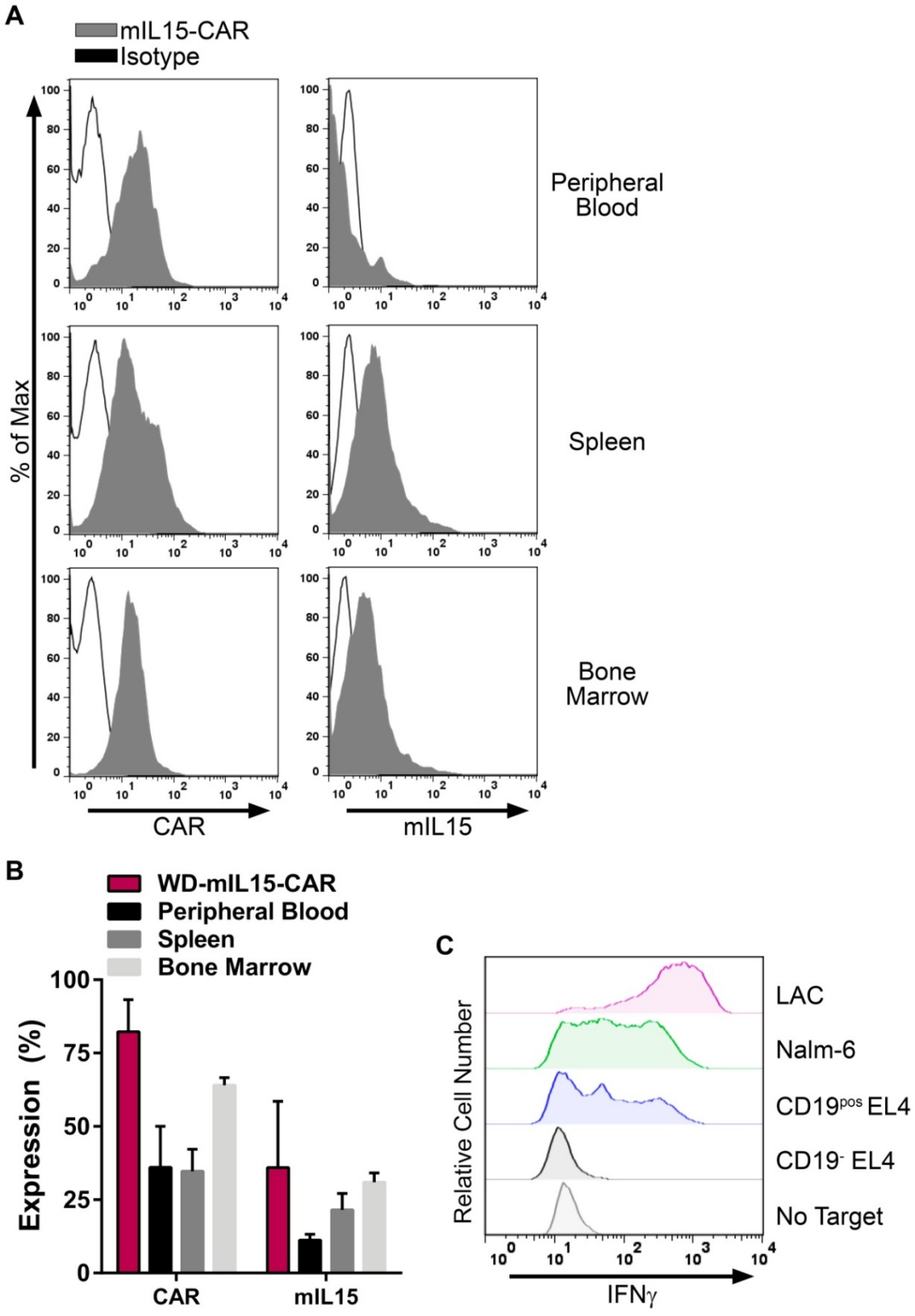
4.3 Discussion

There is sparse information on the maturation fate of cytokine-modified T cells. It has not been elucidated whether such cells can behave in a physiologically relevant manner and modulate their activation to form or maintain memory; or, if they are merely dominated by extrinsic input that generates over-activated T cells in perpetual overdrive. With IL-15's well-known effects on the homeostatic maintenance of T_{CM}, we expected that persisting mIL15-CAR T cells would be characterized primarily as T_{CM}. Contrary to our hypothesis, we did not observe a promotion of that subset. Rather, and most surprisingly, we found the emergence of a low differentiation $CD45RO^{neg}CCR7^{+}$ subset with a memory-like molecular profile and phenotype rarely observed in CAR T cell studies.

Transition from a higher to lower differentiation state of mIL15-CAR T cells after antigen withdrawal was observed on a molecular level (**Fig.25**) and corroborated by phenotypic assessment (**Fig.26**). The modulation of key transcription factors associated with T cell differentiation state (i.e., TCF-1, LEF-1, T-bet, BLIMP-1, EOMES, and Bcl-6) supports the important involvement of intrinsic elements in directing mIL15-CAR T cell fate after antigen withdrawal. That we did not observe intrinsic differences between CAR T cells and mIL15-CAR T cells after *ex vivo* expansion is not entirely surprising. In another study, T cells derived from T_{CM} and T_{EM} subsets both displayed a similar effector phenotype prior to infusion into primates, yet demonstrated differing *in vivo* fitness (50). A plausible explanation for this observation likely involves the dominance of extrinsic factor signaling from CAR-triggering (mimicking TCR and CD28 signaling), costimulatory signaling (i.e. CD27 and CD28), and cytokine signaling (i.e., IL-21 and mIL15 or IL-2) that converge upon

Figure 31. Long-term *in vivo* persisting mIL15-CAR T cells express CAR and remain responsive to TAA. (A) Representative histograms show expression of CAR and mIL15, with CAR detection using the CAR idiotype antibody (clone 136.20.1) and (B) plotted frequencies of marker expression from (A) represented as mean ± sem. Plots and graphs (A,B) show T cell frequencies after gating on human $CD3^+$ and mouse $CD45^-$ cells in the peripheral blood (n = 5), spleen (n = 9), and bone marrow (n = 9). (C) Cells isolated from spleen, liver, or bone marrow were *ex vivo* expanded on aAPC to generate sufficient cell numbers for assessing intracellular IFNγ production in response to $CD19^-$ and $CD19^+$ targets, n = 7 from three tissue sources and four mice. Histograms were gated on CD3.

Figure 31. Long-term *in vivo* persisting mIL15-CAR T cells express CAR and remain responsive to TAA



the PI3K/Akt pathway to modify the cell's molecular profile. In recent years, studies have unraveled that the PI3K/Akt pathway is central to integrating such signaling and acts as a rheostat in T cell differentiation (**Fig. 2**) (25, 27). It has been shown that strong induction of Akt does not sustain CD8 T cell memory, but consequently induces terminal differentiation (26, 27, 172). An additional consideration is the role of epigenetics in T cell memory. It has been shown that knock-out mice of the methyltransferase Dnmt1 or methyl DNA binding protein MBD2 yielded reduced memory T cells and aberrant memory differentiation (173, 174). In T cells, epigenetic changes have been shown to act on cytokine gene loci in effector and memory subsets, and BLIMP-1 can regulate CD8 T cell memory via epigenetic modifications (175-177). This additional level of regulation may offer explanation for our observations regarding the lack of phenotypic differences noted in *ex vivo* expanded T cells, and raises questions as to whether IL-15/mIL15 signaling may contribute to any epigenetic programming.

The finding that *Foxo1* did not increase in parallel with other related pro-memory transcription factors in WD-mIL15-CAR T cells was unanticipated (**Fig. 24**). We found *Il7ra*, *Ccr7*, *Eomes*, *Bach2*, *Bcl-6*, and *Tcf7* to be significantly up-regulated in WD-mIL15-CAR T cells, yet studies have described *Foxo1* to directly or indirectly regulate these genes (127, 163, 167, 168, 178, 179). A possible explanation to account for this paradoxical observation involves *Foxo3*. It was found that *Foxo3* was significantly up-regulated in the WD-mIL15-CAR T cells. The study of FOXOs in T cell memory has been largely assessed with FOXO1 while the role of FOXO3 in this context is not fully understood. Both FOXO1 and FOXO3 integrate into the PI3K/Akt axis of T cell memory regulation, as their activity is inhibited by Akt (178). T cell-specific deletion of *Foxo1* does not result in spontaneous T cell activation, but rather plays a role in regulating genes critical for naïve T cell homeostasis (168). Results from *Foxo3*-deficient mice have been somewhat conflicting, but like *Foxo1* they do not show lymphocytes with spontaneous activation (180). However, it took conditional deletion of *Foxo1*, *Foxo3*, and *Foxo4* in hematopoietic stem cells to induce aberrant cell cycle and apoptosis features (181). These studies suggest that there may be some level of redundancy amongst the FOXOs. The dominant *Foxo3* transcription over *Foxo1* in our system was associated with an increased proportion of the less differentiated $CD45RO^{neg}CCR7^{+}$ subset and is a novel observation. This model may be useful in further examining the roles of FOXO1 and FOXO3 in mature T cells and their regulation in the maintenance of memory. In this study, we observed modulation of

transcript levels of *Foxo1* and *Foxo3* that revealed intriguing results with much potential for elucidating the incompletely defined relationships of this family of transcription factors in mature T cell differentiation and homeostasis. Present understanding of FOXO regulatory mechanisms and instituted gene expression programs indicate that the function of this family of transcription factors is largely in response to alterations in environmental conditions and cellular stress (ie., oxidative stress and AMP-activated protein kinase activity) (182). Moreover, FOXOs are regulated by cytoplasmic shuttling and post-translational modifications (PTMs), including phosphorylation, methylation, ubiquitylation, and acetylation (182). Thus, future investigation into the context-dependent roles of FOXOs in T cells must assess cellular/environmental status along with the various PTMs of FOXOs in the different memory subsets of T cells. Determining whether FOXO3 is involved in the generation or maintenance of the $CD45RO^{neg}CCR7^{+}$ subset would contribute to the limited knowledge of this recently identified subset and, in general, to FOXO3 mechanisms in mature T cell differentiation or homeostasis.

We observed that mIL15-CAR T cells persist in a spectrum of memory states, including a substantial population exhibiting very low differentiation, delineated as $CD45RO^{neg}CCR7^{+}$ (**Fig. 27**). This subset also expressed high levels of CD45RA, CD27, TCF-1, and modest frequency of cells also expressed IL-7Rα and CD62L. A study with IL-15-secreting CAR T cells that evaluated the long-term *in vivo* phenotype of persisting T cells observed increased CD45RA expression and a small subset (~6%) of $CD45RA^{+}CCR7^{+}$ cells in the spleen (92). Though our long-term engraftment was not nearly as high as in the reported study, we observed a 24% average frequency of the $CD45RO^{neg}CCR7^{+}$ subset in the spleen. The caveat being that our T cell manufacture technique uses more rounds of aAPC stimulation, thus likely decreasing memory potential in the cells. Additionally our *in vivo* model was TAA-free whereas the other study assessed T cells from mice that survived tumor challenge. Contrasting our results, antigen-specific T cells modified to express constitutively activate STAT5, and assayed after 20 days *in vivo,* demonstrated an effector phenotype characterized by high levels of T-BET, effector molecule expression, and diminished IL-2 production (183). Yet, other studies examining the mechanisms of STAT5 have shown constitutive STAT5 to promote greater memory populations or supported both memory and effector T cells, which is more in line with our results (154, 184). Polyclonal T_{CM}-derived cells expressing chimeric cytokine receptors signaling through CD127 or CD122 cytoplasmic domains activated STAT5 and resulted in moderate to low expression

of memory associated molecules, respectively (120). However, CD45RA/RO staining was not performed and thus memory subsets could not be defined. These data collectively suggest that (i) providing only STAT5 activity may not necessarily recapitulate the signaling networks that promote a low differentiation state or (ii) the STAT5 activity level was inappropriate for early memory formation or maintenance. Our data showed that mIL15-CAR T cells are representative of several memory subsets and indicated that some of these cells received an appropriate level of mIL15 costimulation to maintain a low differentiation state.

Our T_{SCM}-like population of WD-mIL15-CAR T cells could be described as $CD45RA^{+}CD45RO^{neg}CCR7^{+}CD122^{+}CD95^{+}$ which was similar for the majority of markers to other studies describing T_{SCM} (52, 126) but for the expression level of CD62L and CD28, which was at a lower frequency in our $CD45RO^{neg}CCR7^{+}$ WD-mIL15-CAR T cells. This may indicate that the $CD62L^{neg}CD28^{neg}$ cells within the $CD45RO^{neg}CCR7^{+}$ T cell subset are further along in the differentiation spectrum than T_{SCM} described by other groups (52, 126). The generation of $CD45RO^{neg}CCR7^{+}$ that are $CD62L^{neg}CD28^{neg}$ has, to our knowledge, not been reported and the functional consequences of the lack of these markers in this putative low differentiation subset is unknown. The down-modulation of CD28 may be due to IL-15 costimulation in the mIL15-CAR T cells. IL-15 has been shown to induce proliferation of memory CD8 T cells which resulted in a loss of CD28 in actively dividing T cells *in vitro* (164, 166). While these $CD28^{neg}$ CD8 memory T cells showed no proliferative defects to IL-15 (166), it was also found that IL-21 countered the IL-15-induced down-modulation of CD28 (185). Thus, the addition of IL-21, or a membrane-bound IL-21, could be considered should the preservation of CD28 expression be desired in the mIL15-CAR T cells. CD62L down-modulation was observed in WD-mIL15-CAR T cells, but not to the same extent as CD28. Unlike with CD28, it is unknown whether IL-15 may play a role in this modulation. This observed phenomenon may also be indicative of the complexity of memory T-cell phenotypes and while the $CCR7^{+}CD62L^{neg}$ memory T cell subset is not one of the archetypal memory subsets observed and discussed, this subset has been detected in mice after viral infection (186).

Gattinoni et al. (2011) generated low differentiation T_{SCM} ($CD45RA^{+}CD45RO^{neg}CD62L^{+}$ $CCR7^{+}IL\text{-}7R\alpha^{+}CD95^{+}$) from naïve T cells using a glycogen synthase kinase 3β (GSK-3β) inhibitor to activate the Wnt pathway (52). In contrast, Cieri et al. (2013) generated similar T_{SCM}, albeit $CD45RO^{+}$, using IL-7 and IL-15 (126). Our T_{SCM}-like population of WD-mIL15-

CAR T cells ($CD45RA^+CD45RO^{neg}CCR7^+CD122^+CD95^+$) only had access to IL-15-induced signaling from mIL15. Cieri et al. (2013) reported that IL-15 alone could generate a small population of T_{SCM}, but far superior numbers were generated when IL-7 was used in combination with IL-15 (126). This, however, does support our observation of the promotion of a low differentiation subset solely with IL-15-mediated signaling. Additionally, the inclusion of IL-7-mediated signaling in some manner (i.e., membrane-bound IL-7 expressed on T cells) is a valid consideration for us to augment the development or maintenance of this desirable population in our mIL15-CAR T cell cultures and potentially *in vivo*. We also noted the up-regulated expression of genes related to the Wnt-signaling pathway (*Tcf 7*, *Lef1*, and *Axin2*; **Fig. 24**), which are reported to be regulated by Akt (187). Moreover, since *Axin2* is a direct target of the Wnt pathway through TCF-1 and LEF-1 activity, its up-regulated status suggests that Wnt signaling is active in WD-mIL15-CAR T cells. Akt signal strength is involved with regulating the Wnt pathway where weaker signaling sustains a memory phenotype (26). FOXOs have been shown to interact with β-catenin and could therefore be a contributor to modulating T cell differentiation (188), but this still needs to be further investigated. There is still much to unravel with the cross-talk between the PI3K/Akt and Wnt/β-catenin pathways. Delving into how Wnt signaling may be active in some populations of mIL15-CAR T cells when they receive only IL-15-based signaling instead of exogenous Wnt pathway agonists may help to elucidate this connection. Moreover, the observation of putative Wnt pathway activation in our system indirectly suggests that signaling induced by mIL15 only weakly signals the PI3K/Akt pathway so as to not inhibit the Wnt pathway. Further validation of this observation would contribute to knowledge on how the PI3K/Akt node acts as a rheostat to modulate T cell differentiation. In order to be better able to modulate CAR T cell phenotype and *in vivo* fitness, valuable information may be gained by further unravelling the specific pathway stimulation that is achieved in the T_{SCM}-like mIL15-CAR T cells.

Our data showed the presence of the $CD45RO^{neg}CCR7^+$ population in the peripheral blood and spleen of NSG mice at 50 days after adoptive transfer (**Fig. 30**). In contrast, Gattinoni et al. (2011) adoptively transferred polyclonal T_{SCM} (generated by GSK-3β inhibition) with CD8-depleted PBMC (for human cytokines and costimulation) into NSG mice. At four weeks after transfer, cells that were found in the spleen uniformly showed an effector phenotype (52). This is in contrast to Cieri et al. (2013) where polyclonal T_{SCM} adoptively transferred into a GVHD model maintained high CD62L and low IL-7Ra

expression, but CD45RA^{neg}, on T cells in the peripheral blood at two weeks in primary recipients and one week in secondary recipients (126). It is an interesting observation that T_{SCM} generated via cytokine signaling persisted *in vivo* with a less differentiated phenotype than T_{SCM} produced by pharmacological methods. The mIL15-CAR T cells continued to receive cytokine costimulation, thus it was anticipated that they retain some memory potential in the absence of TAA. But, the T_{SCM} having received IL-7 and IL-15 *in vitro* appeared to carry with them some lasting effects that limited differentiation in comparison to T_{SCM} given GSK-3β inhibition. Determining the mechanism for this disparity (i.e., epigenetics and post-translational modifications) could have clinical implications as pharmacological methods of modulating T cell differentiation have been proposed for use in the clinic, but other more robust approaches may be found. Moreover, if epigenetics or some other post-translational regulatory mechanism is at play with IL-7 and IL-15 signaling, understanding if such processes operate in mIL15-CAR T cells could assist in identifying methods to further manipulate CAR T cell differentiation state.

Homeostatic proliferation, IL-7, and IL-15 have been shown to be integral in generating CD45RA^{+}CCR7^{+} early memory populations (126, 189). We uniquely showed that *in vitro* mIL15, and not soluble IL-2 or IL-15, played a role in preserving the CCR7^{+} subset. It has been demonstrated that differential signaling is induced between soluble IL-15 and transpresented IL-15 (71), and thus it may plausible that the particular surface expression level and specific signaling induced by the membrane-bound or transpresented nature of mIL15 could be providing appropriate costimulatory signaling in CCR7^{+} subsets. Or, it may be a phenomenon of mIL15 providing just the 'right' amount of signaling. Moreover, it is likely that the soluble cytokines present *in vitro* are at supra-physiologic levels that induced strong pathway activation, which is known to result in T cell differentiation (27).

This study assessed the bulk characteristics of cells and thus could not make conclusions regarding lineage relationships between T cell subsets. Studies reporting on the generation of T_{SCM} have indicated their results to be consistent with a linear differentiation model (17, 52, 126). In contrast, it has been reported that CD45RA^{+}CCR7^{+} cells emerged from T_{CM} after culture with IL-7 and IL-15 in the absence of antigenic stimulation (189). In our withdrawal assay, we observed a decline in CD45RO^{+}CCR7^{+} T cell numbers with a concomitant increase of the CD45RO^{neg}CCR7^{+} subset. This may be due to cell death in the CD45RO^{+}CCR7^{+} subset with proliferation in CD45RO^{neg}CCR7^{+} subset. However, that seems like an unlikely outcome considering the numerous studies

reporting that IL-15 is critical to supporting T_{CM} (134, 190). Alternatively, observations by Geginat et al. (2003) showed a proportion of sorted T_{CM} de-differentiated to a $CD45RO^{neg}CCR7^{+}$ in the presence of IL-7 and IL-15 in the absence of antigen (189). Sorting the subpopulations prior to antigen withdrawal may identify which process is at work in our model and contribute to knowledge on the model of T cell differentiation. Moreover, identification of the source subset(s) for T_{SCM} could then fold into methods for developing clinically relevant methods to augment T_{SCM} in the infusion product for CAR T cell therapies. Sorting these subsets and individually testing their antitumor potency with *in vivo* models would contribute knowledge to more clearly identify which T cell subset would possesses the greatest therapeutic potential.

The characterization of mIL15-CAR T cells in this thesis was performed in the absence of TAA. From this we have determined the memory potential of these cells and assembled a profile of the intrinsic factors involved, which had been undefined for long-term persisting CAR T cells. Future directions, though, should next assess mIL15-CAR T cells after tumor challenge to determine how the T cell memory profile has changed and how their *in vivo* fitness changes subsequent to tumor challenge. Much investigation has been conducted on T cell differentiation during infection, but is much less studied in the context of tumor challenge. As discussed in Chapter 3, mIL15 may have more optimal function with other CAR configurations. Assessing how the different CAR configurations alter the mIL15-CAR T cell molecular profile in response to tumor challenge may provide much needed information on optimizing CAR T cell therapy for different tumors or tumor microenvironments (i.e., lack of CD80/86, tumor burden level, lack of CD4 help, and immunosuppression by T_{REGs} and TGF-β).

Nonetheless, while mechanisms for the generation of such early differentiated cells remain to be fully elucidated, we showed that these subsets were promoted in our system via costimulation from mIL15. While more differentiated subsets of WD-mIL15-CAR T cells were found to persist long-term, their *in vivo* functional capacity is anticipated to be poor (22). The preserved memory potential that we observed in our system is an important factor as it has been demonstrated by numerous studies that the greatest therapeutic potential is mediated by the least differentiated T cell subsets (52, 126). While it is currently not feasible to treat patients with naturally occurring T_{SCM}, due to their low circulating frequency, identification of strategies to generate clinically relevant numbers of genetically modified T_{SCM} would greatly contribute to the advancement of CAR T cell-based therapies.

The results presented herein showed that, in our system, mIL15 promoted a T_{SCM}-like population of CAR-modified T cells, and thus bolsters the rationale for using mIL15 in further developing clinically-compliant strategies to augment this desirable T cell subset in immunotherapy infusion products.

CHAPTER 5

General Discussion and Future Directions

5.1 Dissertation Summary

Adoptive transfer of CAR T cells have demonstrated great potential in treating various leukemias, however persistence of infused T cells remains an impediment to advancing therapeutic outcome (43, 63, 77, 78) in the general field as well as with our focus on treating low TAA in MRD. The primary aim of this dissertation was therefore to develop and test a novel cellular immunotherapy approach to resolve this standing clinical issue. Our strategy to address this deficit utilized the pleiotropic effects of IL-15 (85, 86, 191): (i) to support initial survival of infused T cells that are a heterogeneous mixture of differentiation states and (ii) for the long-term maintenance of subsets that remain responsive to IL-15, which are typically less-differentiated and long-lived T cells.

We generated mIL15 to keep the cytokine targeted to responding CAR T cells and to mimic the transpresentation mechanism by which IL-15 functions *in vivo*. Using clinically compliant methods, we demonstrated the generation of mIL15-CAR T cells qualitatively suitable for adoptive transfer. The mIL15-CAR T cells showed enhanced STAT5 costimulation relative to cytokine unsupplemented CAR T cells. Moreover, it was demonstrated that mIL15 could mimic transpresentation and induce STAT5 signaling on CAR T cells that did not express mIL15.

In immunocompromised NSG mice bearing a disseminated $CD19^+$ leukemia, the mIL15-CAR T cells demonstrated both persistence and an antitumor effect, whereas the CAR T cell counterpart could not maintain significant persistence despite the presence of TAA. In a preventative model where CAR and mIL15-CAR T cells were engrafted first followed by the introduction of a disseminated $CD19^+$ leukemia, only the mIL15-CAR T cells were found to persist, eradicate tumor cells, and extend survival. It was also shown that mIL15-CAR T cells were capable of persisting independently of TAA.

It is uncharted territory as to the differentiation status of persisting cytokine-modified CAR T cells. It is unknown what role intrinsic factors may play in supporting these surviving T cells. Since IL-15 was selected, in part, for its critical role in supporting long-lived

memory CD8 T cell subsets, it was hypothesized that T_{CM} subsets would persist preferentially. Characterization of the mIL15-CAR T cells that persisted long-term was in the absence of TAA. *In vitro* assays showed that surviving WD-mIL15-CAR T cells maintained pSTAT5 activity and Bcl-2 levels whereas control CAR T cells showed diminished levels of these molecules indicating that mIL15 was still functional. Comparing the long-term persisting WD-mIL15-CAR T cells to the earlier *ex vivo* expanded mIL15-CAR T cells, it was noted that WD-mIL15-CAR T cells significantly expressed genes associated with a less differentiated state. This was validated by flow cytometry for specific memory and differentiation-related transcription factors and cell-surface markers. Moreover, WD-mIL15-CAR T cells acquired the capability to produce IL-2, a hallmark of less differentiated memory cells. Assessing the subset composition, it was revealed that WD-mIL15-CAR T cells persisted preferentially as the least differentiated ($CD45RO^{neg}CCR7^{+}$) and most differentiated ($CD45RO^{+}CCR7^{neg}$) subsets. The $CD45RO^{neg}CCR7^{+}$ subset would classically be known as T_N. However WD-mIL15-CAR T cells in that subset were found to be $CD95^{+}$ and $CD122^{+}$, both markers associated with antigen-experienced T cells and T_{SCM}. Therefore, the molecular profile and phenotypic assessment of the bulk population suggested that some cells within make up a T_{SCM}-like subset that is less differentiated than T_{CM}. This *in vitro* WD-mIL15-CAR T cell subset composition was recapitulated *in vivo*, but was tissue-dependent. It was found that WD-mIL15-CAR T cells persisting in the bone marrow took on a more activated phenotype of $CD45RO^{+}CCR7^{neg}$ whilst the peripheral blood and spleen contained the highest frequencies of the $CD45RO^{neg}CCR7^{+}$ subset. This capacity to sustain a less differentiated memory potential *in vivo* further reinforces their suitability for use in the clinic. This also brings to light the importance of determining the mechanism of generation and maintenance of such T cell subsets as this knowledge could ultimately be harnessed for the generation of optimal T cell subsets for ACT. The results in this dissertation provide pre-clinical data that can now be translated into a clinical trial to evaluate mIL15-CAR T cell persistence and disease-free survival of post-HSCT patients with MRD.

5.2 Treating MRD: A Focus on Forming Durable Immunologic Memory

At the genesis of this project, the primary aim was to achieve CAR T cell persistence under low TAA so that it may translate to the clinic for treating patients with MRD or

sequestered TAA. We achieved this with the mIL15-CAR T cells (**Figs. 15,19**). A treatment approach was developed for a subset of patients that needed improved treatment options. However, with results from clinical trials and observations from this study, we propose that treating MRD may be a more desirable approach over treating bulky disease for several reasons.

First, it may make T cell therapy safer. Clinical trials treating bulky disease, while achieving some remarkable results with complete remissions, have also had severe treatment related toxicities, including tumor lysis syndrome and cytokine storms (77, 145, 192). Such toxicities can be correlated to the level of tumor burden at time of infusion (78). Hence, treating MRD would be anticipated to have a lower magnitude of toxicities.

Second, lower tumor burden may allow for the establishment of immunologic memory and durable CAR T cell immunosurveillance for extended protection against relapse. In the successful study by Kalos et al., T cells were detected persisting in patients after high tumor burden was eradicated (77). And while CD8 CAR^{neg} T cells displayed some markers associated with memory, CD8 CAR^{+} T cells did not express any of these markers other than low levels of CD27. In general, the CAR^{+} T cells displayed a highly differentiated T_{EMRA} phenotype (145). It is well accepted that repetitive antigen exposure and exposure to strong stimuli, (i.e., strong TCR stimulation and inflammatory cytokines) leads to progressive differentiation and loss of function (21, 22, 25, 193). Therefore, the low-TAA environment associated with MRD may foster a more pro-memory environment, in the sense of a lower magnitude of antigenic stimulation, less repetitive stimulation, and the generation of lower levels of inflammatory cytokines. With mIL15-CAR T cells able to persist under low TAA, there is potential for CAR T cells to reside as memory cells after tumor is cleared and to maintain immunosurveillance for durable protection against relapse.

5.3 Application to Other CARs and Cell Therapies

The use of mIL15 is, by no means, limited to the CD19-specific CAR. Moving mIL15 into therapies treating solid tumors could provide assistance not only with persistence, but also against tumor microenvironment suppressive factors, for which solid tumors are notorious (194). IL-15 has been shown to counter the immunosuppressive effects of transforming growth factor-β (195, 196), as well as regulatory T cells (197, 198). Hence,

mIL15 could be paired with most any other CAR targeting solid tumors, however the most logical target to move to first may be melanoma. IL-15 has recently entered into the clinic and its first implementation was in the treatment of melanoma, either as a monotherapy (NCT01946789) or infused with autologous lymphocytes (NCT01369888). Murine models with B16 revealed superior antitumor effect with the use of a soluble IL-15/IL-15Rα sushi domain fusion protein (RLI) (199) or antibody fusion protein (98). A CAR targeting melanoma is toward the MAGE1 antigen (200). Preclinical data using RLI (a soluble IL-15/IL-15Rα hyperagonist) also showed significantly enhanced antitumor activity against colorectal cancer (199) and a targeted form specific for GD2 (201), which is found on neuroblastoma. CARs targeting these tumors are in clinical trials (36) and could thus be readily translated to trials including mIL15. The utility of mIL15 is diverse, but these solid tumor targets are suggested to be the most promising.

5.4 Combination Therapies

5.4.1. Combining mIL15-CAR T Cells and Small Molecule Inhibitors

Cancer cells may overexpress anti-apoptotic proteins such as Bcl-2 or possess persistently activated pathways (i.e., STAT5 and STAT3) that result in aberrantly high resistance to natural apoptotic mediators or pharmacologic agents. Combining the approaches of drugs and immunotherapy to utilize more than one apoptotic pathway could increase tumor cell death and decrease therapeutic resistance. The use of small molecule inhibitors may shift the balance enough between the pro- and anti-apoptotic molecules within tumor cells to sensitize them toward death or decrease resistance to killing during encounter with tumor-specific T cells.

Elevated Bcl-2 expression has been found in hematologic malignancies [i.e., acute myelogenous leukemia (AML) and chronic lymphocytic leukemia (CLL)] and solid tumors (i.e., melanoma, breast and ovarian cancer) (202-206). Persistently activated STAT3 or STAT5 has been observed in AML, CLL, chronic myelogenous leukemia, large granular lymphocytic leukemia, and leukemia cell lines (207-210). Small molecule inhibitors targeting Bcl-2, STAT3, or STAT5 have shown efficacy in inducing apoptosis (207, 208, 211, 212). Moreover, the addition of a Bcl-2 inhibitor to CAR T cells in co-culture with lymphoma and leukemia cells enhanced T cell-induced apoptosis (213). However, the

inhibitor also caused T cells to undergo some level of apoptosis, which may pose a problem for clinical application.

In our study, we showed that pSTAT5 and Bcl-2 levels were sustained by mIL15-CAR T cells (**Fig. 23**). This would suggest that mIL15-CAR T cells may have some level of resistance to the apoptotic-inducing effects of these small molecule inhibitors of this pathway, and indeed they may. The mIL15-CAR T cells were able to resist pSTAT5 abrogation by a STAT5 inhibitor that CAR T cells could not (L. Hurton, unpublished observation). Thus, this may be a viable complementary approach that could be used to boost antitumor activity by mIL15-CAR T cells.

5.4.2. Combining Cell Therapies

Single agent treatment of cancer is not always successful in the long run. The cancer cells find a way to evade complete eradication and the patients relapse in time. A multipronged approach should be implemented, thus increasing therapeutic potential. Physiologically, T cells and NK cells function coordinately in an orchestrated immune response to eliminate the pathogen. NK cell therapies are entering into the clinic for various malignancies. Moreover, they can be reliably and significantly expanded *ex vivo to* obtain sufficient numbers for adoptive transfer (214). Combining CAR T cells with NK cells in adoptive immunotherapy may be advantageous to diversify the antitumor response. Rather than targeting a single antigen, the innate activities of the NK cells may allow for epitope spreading leading to a more well-rounded response that could plausibly decrease the risk of tumor antigen escape (215). Another aspect of the combination therapy could be NK-promoting effects from the T-cell transpresented mIL15. Though a recent study suggested that the homeostasis of human NK cells in not dependent on IL-15 (216), other work on human NK cells indicates otherwise. Huntington et al. demonstrated that IL-15/IL-15Rα complexes, but not monomeric IL-15, induced extensive human NK cell proliferation (217). In a clinical trial, it was reported that after multiple doses of IL-15, NK cells were found to increase 4 to 10 fold (218). Moreover, we observed massive expansions of NK cells in cultures that only had mIL15 cytokine availability (**Fig. 8)**. Moreover, we demonstrated that mIL15 can be transpresented and induce cytokine signaling in another cell (**Fig. 10**). Together, this would suggest that a combination therapy using *ex vivo* NK cells and mIL15-CAR T cells may mediate superior antitumor effects.

5.5 Clinical Safety

In humans, dysregulated IL-15 production, elevated serum levels, or abnormal IL-15 signaling is associated with autoimmune diseases and may be involved in the pathogenesis of T-cell LGL leukemia (219, 220). Moreover, transgenic mice engineered to maximally overexpress IL-15 develop leukemia (221). It has also been reported that one IL-15-transduced T cell culture resulted in a clonal expansion, though the etiology was not definitively identified (222). In contrast, constitutive STAT5 expression in lymphocytes did not prompt transformational events (135, 154). Similarly, our study demonstrated enhanced engraftment of T cells without any overt dysregulated proliferation or activation *in vitro* or *in vivo*. The molecular profiling by NanoString showed the down-regulation of activation genes (i.e., IL-2Rα and CD38; **Fig. 24**) and we did not observe any splenomegaly in mIL15-CAR treated mice in the preventative or TAA-free mouse models (L. Hurton, unpublished observations). Despite the promising therapeutic potential of mIL15-CAR T cells, concerns about the long-term safety of T cells receiving chronic IL-15 signaling are justified and must be addressed for clinical implementation. A clinically used approach to conditionally ablate genetically-modified T cell is by additional co-modification of the cells with an inducible caspase-9 suicide gene. Exposure to a dimerizer drug effectively induces apoptosis in the modified T cells (93, 223).

5.6 Clinical Implications

Clinical trials will now be addressing the targeting of minimal burdens of $CD19^+$ malignancy as patients are enrolled earlier in their disease course and receive concomitant chemotherapy. The intended goal of mIL15 was to enhance T-cell costimulation *in situ* to support T-cell persistence independently of CAR activation, to sustain antitumor activity in a low TAA environment, and preserve T cell memory potential. We have demonstrated that mIL15-CAR T cells can stand up to the challenge of a TAA-poor environment. Their use in the clinic should not require the use of exogenous IL-2, thereby improving the safety profile in that regard. In comparison to trials using CAR T cells to treat bulky disease, it is anticipated that there will be fewer events of tumor lysis syndrome and cytokine storm since mIL15-CAR T cells will be infused under low bio-burden conditions. Finally, mIL15-CAR T cells were specifically generated to possess enhanced persistence after adoptive transfer.

This should translate into establishing durable immunologic memory that would enable extended immunosurveillance resulting in improved disease-free survival of MRD patients.

CHAPTER 6

Materials and Methods

6.1 Plasmids

6.1.1 mIL15 (IL-15/IL15-Rα fusion) Transposon

The mIL15 construct fuses the IL-15 cDNA sequence (NM_000585.4) to the full-length IL-15Ra (NM_002189.3) with a glycine-serine linker ($SG_3SG_4SG_4SG_4SG_3$ SLQ) and has a C-terminal FLAG (x3) epitope tag (DYKDDDDK). In line with these reports, the signal peptides for IL-15 and IL-15Rα were omitted and the IgE signal peptide (gb|AAB59424.1) was used for the mIL15 fusion protein. This construct will produce an IL-15 that is membrane-bound, but also presented in the context of IL-15Rα. Mortier et al. created a similar IL-15 fusion protein, albeit in a secretable configuration, and demonstrated that this fusion protein with a covalent linker was functional. Moreover, this construction had increased potency over non-covalently linked IL-15 and soluble IL-15Rα (103). The mIL15 DNA sequence was codon-optimized for expression in human cells and the DNA plasmid, IL15-IL15Ra-FLAG_pMK-RQ, was synthesized by GeneArt (Regensburg, Germany). IL15-IL15Ra-FLAG_pMK-RQ and GlySer-EGFP-mIgG1/pSBSO underwent digestion with Nhe and Xho restriction enzymes to obtain the mIL15 insert and pSBSO vector backbone respectively. The resulting fragments were excised and gel purified (AIAquick Gel Extraction Kit, Quagen) and subsequently ligated using T4 DNA ligase (Promega) yielding IL15-IL15Rα-Flag/pSBSO. Transformation of C2925 bacteria (Invitrogen) with the plasmid and selecting clones for amplification, both under kanamycin drug selection, was followed by large-scale amplification plasmid DNA. The DNA was purified with EndoFree Plasmid Maxi Kit (Quiagen). Prior to use in cell transfection, the plasmid was validated by analytical digestion and had a spectrophotometer A_{260}/A_{280} value of 1.80 – 2.00.

6.1.2 CD19-specific CAR Transposons

Generation of the SB transposon encoding the second generation CD19-specific CAR (CD19RCD28/pSBSO) under the human elongation factor-1α promoter has been

previously described (224). A CD19-specific CAR co-expressing an enhanced *ffLuc*, designated CAR-*ffLuc*, (CD19RCD28-T2A-Flag-*ffLuc*/pT2SBSO) was used for *in vivo* monitoring of T cells by BLI. Generation of the plasmid (produced by S. Olivares) is as follows. *Sleeping beauty* plasmid CD19RCD28mz-T2A-Flag-FFluc (CoOp)/pT2SBSO, co-expressing the human codon optimized CD19-specific CAR and the luciferase reporter gene from firefly (*Photinus pyralis)* via a self-cleaving 2A peptide from *Thosea asigna* virus, was derived from the CD19RCD28mz-T2A-hΔTK (CoOp)/pΔSBSO plasmid. The *sleeping beauty* inverted-direct repeats (IRs/DRs) T1 were replaced with the hyperactive IRs-DRs T2, to improve transposition, and the firefly luciferase reporter gene replaced the hΔTK transgene. In the first step, plasmids CD19RCD28mz-T2A-hΔTK (CoOp)/pΔSBSO and CD19RCD28mZ (CoOp)/pT2SBSO were digested with *StuI-KpnI* and *ClaI-KpnI* restriction enzymes, respectively, with an extra blunting reaction after the *ClaI* digestion in order to create compatible blunted ends. The resulting fragments were purified by gel electrophoresis and ligated using T4 DNA ligase to create intermediate plasmid-I, CD19RCD28mz-T2A-hΔTK (CoOp)/pT2SBSO. The second step consisted of replacing the T2A-hΔTK transgene with a T2A-flag tag fusion and a more suitable multiple cloning site to facilitate the cloning of the reporter gene. This T2A-flag-MCS was created by polymerase chain reaction (PCR) primer annealing and TOPO-TA cloning for validation of the sequence. Once the sequence was validated, plasmids CD19RCD28mz-T2A-hΔTK (CoOp)/pT2SBSO and T2A-Flag-MCS/TOPO were digested with *BspE1* and *SpeI* restriction enzymes. The resulting fragments were purified by gel electrophoresis and ligated using T4 DNA ligase to create intermediate plasmid-II, CD19RCD28mz-T2A-Flag (CoOp)/pT2SBSO. The final step in the process was to introduce the firefly luciferase transgene in-frame with the flag-tag. The firefly luciferase gene was digested out from the previously made plasmid Flag-FFLuc-HyTK (CoOp)/pT2SBSO using *SnaBI* and *EcoRV* restriction enzymes. The same restriction enzymes were used to digest the accepting vector followed by a dephosphorylation reaction to prevent vector self-ligation due to the resulting compatible blunt ends. The resulting fragments were purified by gel electrophoresis and ligated using T4 DNA ligase to create the final plasmid, CD19RCD28mz-T2A-Flag-FFluc (CoOp)/pT2SBSO.

6.1.3 SB11 Transposase

The hyperactive transposase (SB11) is under the control of a cytomegalovirus (CMV) promoter in the pKan-CMV-SB11 SB transposase as previously described (224).

6.1.4 Truncated CD19 Transposon

A truncated CD19 plasmid (dCD19-F2A-Neo/pSBSO) was constructed by cloning the extracellular and transmembrane domains of codon-optimized human CD19 (Accession: M84371) from a GeneArt generated vector into a pSBSO plasmid linking with neomycin resistance via a F2A self-cleavable peptide sequence (performed by S. Olivares).

6.1.5 *rLuc*-mKate Reporter

The *rLuc*-mKate reporter (*rLuc*8.6-535-T2A-mKateS158A/pLV430G) is a lentiviral vector used for modifying tumor cell lines used for imaging tumor burden during *in vivo* experiments. The sequences for *rLuc*8.6-535 and mKateS158A were derived previous descriptions (225, 226), codon optimized as previously reported (227) and synthesized by GeneArt (Regensburg, Germany) as a B1 *rLuc*8.6-535-T2A-mKateS158A B2 construct. The B1 and B2 sites are lambda phage recombination sites used in Gateway Recombination Cloning Technology (Invitrogen) synthesizing the construct via BP reaction into pDONR221. Subsequently, *rLuc*8.6-535-T2A-mKateS158A/ pDONR221 was recombined into pLV430G (228), a self-inactivating lentivirus encoding an internal U3 promoter from murine stem cell virus (Performed by B. Rabinovich). Lentiviral particles were packaged by transfecting 293METR cells with *rLuc*8.6-535-T2A-mKateS158A/pLV430G using Lipofectamine 2000 (Invitrogen). Virus was harvested 48 hours post-transfection and concentrated using 100kDa NMWL filters (Millipore) to yield the final viral particle supernatant used for transductions (performed by J. Krishnamurthy).

6.2 Cell Lines and Their Propagation

All cell lines in this study were maintained in complete media [RPMI 1640, 10% heat-inactivated fetal bovine serum (Hyclone), and 1% Glutamax-100 (Gibco)] in humidified conditions at 5% CO_2 and 37˚C.

6.2.1 K562-derived aAPC (Clone 9)

K562-derived aAPC (Clone 9) were acquired from the University of Pennsylvania courtesy of Dr. Carl June and were generated by retroviral transduction with CD64, CD86, CD137L, and truncated CD19 as described previously (106). Validation of co-expression of

antigen and co-stimulatory molecules (CD19, CD86, and CD137L) were performed before addition to T-cell cultures.

6.2.2 NALM-6 and *rLuc*$^{+}$mKate^{+}NALM-6

NALM-6, a pre-B cell leukemia, was obtained from American Type Culture Collection (Manassas, VA). Generation of the *rLuc*$^{+}$mKate^{+}NALM-6 cells underwent lentiviral transduction with viral particles encoding *rLuc* and mKate (from vector *rLuc*8.6-535-T2A-mKateS158A/pLV430G) by plating NALM-6 cells with the viral particles and polybrene (Millipore) followed by centrifuging plates at 2000rpm for 1.5 hours. After outgrowth of transduced cells, mKate^{+} cells were subsequently FACS Aria (BD Biosciences) sorted (performed by J. Krishnamurthy).

6.2.3 EL4 and dCD19^{+}EL4

The EL4 cell line, a murine T-lymphoma, was acquired from ATCC. The dCD19^{+}EL4 cells were generated by electro-transferring the dCD19 and SB11 plasmids into EL4 using the Amaxa mouse T cell nucleofector kit (cat.no. VPA-1006; Lonza, Basel, Switzerland) with subsequent selection under 0.8mg/ml G418 (InvivoGen, San Diego, CA) in culture media (performed by T. Mi).

6.3 Generation and *Ex Vivo* Expansion of mIL15 and CAR-modified T Cells

Anonymized normal human donor blood bank buffy coats were obtained from the Gulf Coast Regional Blood Center (Houston, TX) and mononuclear cells were isolated by Ficoll-Paque density gradient centrifugation (GE Healthcare) followed by cryopreservation. The mIL15$^{+/-}$CAR^{+} T cells were generated and propagated based on modified standard operating protocols used to generate clinical-grade CD19-specific T cells using the *Sleeping Beauty* system (105, 229) and depicted in **Figure 2**. Cryopreserved PBMC were thawed the day of electroporation (day 0) and rested for 2-3 hours in complete RPMI media. Viable cells were enumerated based on Trypan blue exclusion using a Cellometer automated cell counter (Auto T4 Cell Counter, Nexcelom Bioscience) with the 'PBMC_human_frozen' program. A total of 2 x 10^{7} live PBMC were resuspended with selected DNA plasmids in 100μl of human T cell nucleofector solution (Human T cell Kit, Lonza) were used per electroporation reaction. Electroporation reactions for CAR-

modification of cells contained 15μg of CAR transposon (CD19RCD28/pSBSO) and 5 μg of SB11 transposase (pKan-CMV-SB11) DNA. Electroporation reactions for mIL15 and CAR co-modification of cells contained 7.5μg of mIL15 transposon (IL15-IL15Rα-Flag/pSBSO), 7.5μg of CAR transposon (CD19RCD28/pSBSO), and 5 μg of SB11 transposase (pKan-CMV-SB11) DNA. PBMC that were modified for *in vivo* experiments instead used the CAR-*ffLuc* (CD19RCD28-T2A-Flag-*ffLuc*/pT2SBSO) transposon in the same quantities that were used for the CAR transposon. Each complete reaction was transferred to a single cuvette and electroporated with program U-14 using Nucleofector II (Lonza). Transfected cells were transferred to a 6-well plate containing phenol-free RPMI supplemented with 20% FBS and 1x Glutamax-100. After resting the cells for 2-3 hours a half media change was performed with fresh phenol-free media supplemented with 1x Glutamax-100 to achieve a final supplementation of 10% FBS, in which the cells were cultured overnight. On day 1, electroporated cells were phenotyped and stimulated with γ-irradiated (100Gy) K562 (Clone 9) aAPC based on their CAR expression with additional aAPC stimulations every 10 ± 2 days. Co-cultures used a ratio of 1:2 CAR^+ T cells to aAPC and were maintained in complete media. Co-cultures were supplemented with only human IL-21 (30ng/ml, PeproTech) during the first week (given 3 times), after which mIL15-CAR T cell cultures continued to receive only IL-21 while CAR T cells received IL-21 and either human IL-2 (50U/ml, Aldeleukin; Novartis, Switzerland) or a soluble IL-15/IL15-Ra-Fc complex (5ng/ml) 3 times per week. The IL-15/IL15-Rα-Fc complex, referred to as IL-15 complex, is a 2:1 molar ratio combination of human IL-15 (PeproTech) and human IL-15Rα-Fc chimera (R&D Systems) containing 5ng/ml of IL-15, thus dosing of IL-15 is denoted by its IL-15 content since not all of the cytokine is in complexed form. This formulation was used as it has been reported that complexing IL-15 with IL-15Rα increases the potency of IL-15 (96, 97). Some cytokine supplementation was modified for specific assays and conditions are defined in other sections. The cultures were monitored for excessive outgrowth of $CD3^-CD56^+$ NK cells, which if exceeding 15% were depleted from the cultures using CD56 microbeads (Miltenyi Biotec) and LS columns (Miltenyi Biotec) per the manufacturer's instructions. Viable T cells were enumerated at the end of each aAPC stimulation cycle (Stim) based on Trypan blue exclusion using a hemocytometer for the first Stim and Cellometer counts ('activated T cell' program) for subsequent Stims. Inferred CAR^+ T cell numbers were calculated by multiplying the $CD3^+CAR^+$ cell fold expansion from the first Stim with the relative starting cell number which would then be multiplied by the CAR-specific T cell expansion from subsequent Stims.

6.4 Immunophenotyping

Up to 1 x 10^6 were stained with fluorochrome-chrome conjugated antibodies: fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein conjugated to cyanine dye (PerCP-Cy5.5), allophycocyanin (APC), AlexaFluor-488, and AlexaFluor-647, which unless otherwise stated, were obtained from BD Biosciences. Unless otherwise stated, antibodies were used at 1:20, 1:40, 1:25, 1:33, 1:20, and 1:20 dilutions, respectively, in a 100μl volume of buffer. Staining for cell surface markers and corresponding Isotype controls was conducted in FACS buffer [phosphate-buffered saline (PBS), 2% fetal bovine serum (FBS), 0.1% sodium azide) for 30 minutes at 4˚C. Intracellular staining was done following fixation and permeabilization for 20 minutes at 4˚C with BD Cytofix/Cytoperm with antibodies diluted in 1x Perm/Wash buffer for 30 minutes at 4˚C (BD Biosciences). Annexin V staining was conducted in 1x annexin binding buffer (BD biosciences). Antibodies used for immunostaining were against human: Bcl-2 (1:5 dilution, clone Bcl-2/100), Fc (to detect CAR; clone H10104, Invitrogen), IL-15 (1:6.7 dilution for APC; clone 34559, R&D Systems), BLIMP-1 (clone 646702, R&D Systems), CAR idiotype (clone 136.20.1)(230), CD3 (clone SK7), CD4 (cloneRPA-T4), CD8 (clone SK1), CD25 (clone M-A251), CD27 (clone L128), CD28 (clone L293), CD19 (clone HIB19), CD45RA (clone HI100), CD45RO (clone UCHL1), CD56 (clone B159), CD62L (clone DREG-56), CD122 (clone Mik-β3), CD127 (1:8.3 dilution; clone 40131, R&D Systems), CCR7 (1:45 dilution for PerCP-Cy5.5; clone G043H7, BioLegend), IL-2 (clone MQ1-17H12), IFNγ (clone B27), pSTAT5 (pY694) [1:5 dilution; clone 47/Stat5(pY694)], T-BET (1:20 for PerCP-Cy5.5; clone 4B10, BioLegend), and unconjugated rabbit anti-TCF1 (1:20 dilution; clone C725.7, Thermo Scientific). Polyclonal goat anti-rabbit IgG-DyLight650 (1:100 dilution; Thermo Scientific) was used as a secondary antibody to detect TCF1. Annexin V (1:20 dilution; BD Bioscience) and anti-FLAG (1:200 dilution; Prozyme) were also used. Data acquisition was on a FACSCalibur using Cell Quest version 3.3 (BD Biosciences). Analyses were with FlowJo software (version 7.6.5, TreeStar).

6.5 Validation of pSTAT5 Induction by mIL15

Ex vivo expanded T cells were assessed at > 10 days after the 4th aAPC stimulation. Cells were washed in PBS to remove residual cytokines from culture media. The cells were then transferred to fresh serum-free culture medium (RPMI supplemented with 1% Glutamax) at a high density of 2 x 10^6/ml and serum and exogenous cytokine starved for 12 hours. Starved CAR T cells indicate baseline or control levels of pSTAT5 while CAR T cells treated with 50-100 ng/ml IL-15 one hour prior to fixation served as the positive control. The cells were pelleted to remove the supernatant, then immediately fixed with 6ml of 37˚C 1x PhosFlow Lyse/Fix Buffer (BD Biosciences), and further incubated in a water bath for 15 minutes at 37˚C. The cells were pelleted again to remove the supernatant, then permeabilized by adding 3ml of 4˚C BD PhosFlow Perm Buffer III (BD Biosciences) while vortexing, incubated on ice for 20 minutes, and then washed with FACS buffer. Cells were stained with pSTAT5 (pY694) antibody for 20 minutes at room temperature in the dark, washed and resuspended in FACS buffer for flow cytometry analysis. For assessing transpresentation of mIL15, CAR and mIL15-CAR T cells were co-cultured at a 1:4 ratio for 16 hours in serum and exogenous cytokine free medium and then processed as previously described.

6.6 Chromium Release Assay

The antigen-specific cytolytic activity of T cells was assessed in a standard 4-hour CRA, as previously described (231). Genetically modified T cells were incubated with 2 x 10^5 ^{51}Cr-labeled target cells (EL4, CD19$^+$EL4, or NALM-6) in V-bottomed 96-well plates (Costar). The percentage of specific lysis was calculated from the release of ^{51}Cr, as previously described, using a TopCount NXT (Perkin-Elmer) (231).

6.7 Intracellular Cytokine Production

Production of cytokines was assessed by intracellular cytokine staining of T cells. T cells were co-cultured with CD19$^+$ or negative targets (EL4, CD19$^+$EL4, or NALM-6) at an effector to target ratio of 1:5 in 200μl of culture media in a round bottom, 96-well plate. T cells were incubated alone as a negative control and with the addition of LAC [PMA

(phorbol 12 myristate 13 acetate) and ionomycin; BD Biosciences) as a positive control. Brefeldin-A (GolgiPlug, BD Biosciences) was added to all cultures to block exocytosis of cytokines produced in response to antigenic stimulation. Cultures were incubated for 6 hours at 37˚C and then stained for surface markers followed by fixation and permeabilization and staining for intracellular proteins.

6.8 *In Vivo* Experiments Assessing T Cell Persistence and Antitumor Activity

Human tumor xenograft models were performed under the approved protocol of the Institutional Animal Care and Use Committee at MDACC. All *in vivo* murine experiments used age and sex-matched humanized NSG mice (NOD.Cg-Prkdc^{scid}IL2rγ^{tm1Wjl}/SzJ, The Jackson Laboratory).

6.8.1 *In Vivo* Bioluminescence Imaging

Serial non-invasive bioluminescence imaging (BLI) was performed to measure persistence of mIL15-CAR and CAR T cells co-expressing *ffLuc*. BLI for *ffLuc* activity was acquired 10 minutes after subcutaneous administration of D-Luciferin potassium salt (215μg/mouse, Caliper) as has been previously described (53, 232). Serial non-invasive BLI was also done to monitor tumor burden of *rLuc*$^{+}$NALM-6 cells. BLI for *rLuc* activity was acquired 20 minutes after intraperitoneal injection of freshly diluted Enduren Live Cell Substrate (68μg/mouse, Promega) as has been previously described (232). Imaging was performed on anesthetized mice in an anterior posterior position using Xenogen IVIS 100 series system (Caliper Life Sciences). Imaging for T cells and tumor were performed on alternate days. T cell and tumor flux (measured as photons/s/cm^2/steradian) were quantified by measuring photon signal within a delineated region of interest (ROI) which encompassed the entire mouse, except the tail. An addition ROI was established for T cell flux, termed 'femur ROI' and encompassed the femur, tibia, and foot region of the mouse. Living Image software (v2.50, Xenogen; Caliper Life Sciences) was used to process the BLI data.

6.8.2 Tissue Preparation

At the end of some experiments, mice were euthanized by cervical dislocation and peripheral blood and tissues were harvested (spleen and bone marrow). Spleens were

manually homogenized in 2% FBS/PBS and passed through a 40μm cell strainer (BD BioSciences). To obtain the bone marrow, the femurs were flushed with 2% fetal bovine serum in phosphate buffered saline (FBS/PBS) using 30G needles (BD Biosciences). ACK lysing buffer (Gibco-Invitrogen) was used to lyse red blood cells in all samples followed by washes with 2% FBS/PBS. Cells were stained for the presence of T cells (CD3), tumor (CD19), and murine lymphocytes (CD45).

6.8.3 Persistence of CAR and mIL15-CAR T Cells in a High Tumor Burden Model

On day -1, mice were intravenously (i.v.) injected via tail vein with 1 x 10^5 NALM-6 cells to establish a disseminated leukemia model. On day 0, mice (n = 5/group) in two treatment cohorts received mIL15$^+$CAR$^+$*ffLuc*$^+$ T cells or CAR$^+$*ffLuc*$^+$ T cells (cultured with IL-2 and IL-21) injected i.v. at 2 x 10^7 CAR$^+$ T cells per mouse. T cell persistence (*ffLuc*) was longitudinally monitored by BLI until day 14 when mice were sacrificed and tissues were harvested for analysis of CD3 by flow cytometry.

6.8.4 Persistence and Efficacy of CAR and mIL15-CAR T Cells in a Low Tumor Burden Model

T cells were engrafted first in this preventative tumor model mimicking MRD, where on day 0 two treatment cohorts (n = 5/group) were i.v. injected with 2 x 10^7 CAR$^+$ T cells: (i) mIL15$^+$CAR$^+$*ffLuc*$^+$ T cells and (ii) CAR$^+$*ffLuc*$^+$ T cells. On day 6, 1 x 10^4 *rLuc*$^+$NALM-6 were i.v. injected. T cell and tumor BLI were monitored until death or sacrifice (day 36) when tissues were harvested for analysis of CD3 and CD19 by flow cytometry. To assess survival over a longer duration, another experiment was conducted in a similar fashion with survival as the experimental endpoint followed to 98 days and tested the following cohorts: (i) mIL15$^+$CAR$^+$*ffLuc*$^+$ T cells plus NALM-6 (n = 7), (ii) CAR$^+$*ffLuc*$^+$ T cells plus NALM-6 (n = 8, cultured with IL-2 and IL-21), and (iii) NALM-6 only. As in the previous experiment, NALM-6 cells were i.v. injected after T cell engraftment at 1 x 10^4 cells per mouse. T cell persistence was followed by BLI until mortality or euthanasia occurred. Mice were considered at the experimental endpoint if they developed hind-limb paralysis or were otherwise moribund.

6.8.5 *In Vivo* Persistence of CAR and mIL15-CAR T Cells in the Absence of TAA

To test T cell persistence in the absence of antigen, 2 x 10^7 CAR$^+$ T cells were i.v. injected into mice in two treatment cohorts (n = 5/group): (i) mIL15$^+$CAR$^+$*ffLuc*$^+$ T cells and

(ii) CAR^{+}*ffLuc*$^{+}$ T cells. BLI was performed to monitor T cell persistence until sacrifice at days 48. Tissues were harvested for analysis of CD3 and CD19 by flow cytometry. Some spleen, liver, and bone marrow samples were *ex vivo* expanded on K562 (Clone 9) aAPCs at an initial stimulation one isolated cell to 5 aAPCs with subsequent stimulations at 1:1. Expanded cells were tested for antigen-specific function by assessing IFNγ production in response to tumor cell targets, as described in section 6.7.

6.9 Assays Characterizing Long-term mIL15-CAR T Cell Persistence in the Absence of TAA

6.9.1 *In Vitro* Phenotypic and Functional Characterization

Persistence and function of T cells after antigen and exogenous cytokine withdrawal were tested using genetically-modified T cells that had undergone four aAPC stimulation cycles. After the fourth Stim, the T cell cultures continued to be maintained with exogenous cytokine supplementation, as described in section 6.3, for 10 days after which T cells were enumerated and transferred to fresh complete RPMI to establish the following treatment groups for the withdrawal assay: (i) mIL15-CAR T cells, (ii) CAR T cells, (iii) CAR T cells supplemented with IL-15 complex (5ng/ml, 2x/week). Cells were enumerated at days 15, 30, 50, and 65. T cells persisting > 65 days after antigen withdrawal, referred to as 'WD-T cells', were then assessed for mIL15 and CAR expression. To verify that mIL15 was functional, pSTAT5 and Bcl-2 levels were assessed in WD-T cells per phosflow and intracellular staining described in sections 6.5 and 6.7, respectively. To test whether the WD-T cells were still functional and responsive to antigen stimulation, they were challenged with CD19$^{+/-}$ targets and IFNγ production was measured by intracellular cytokine staining as previously described in section 6.7. IL-2 production was assessed upon stimulation with LAC and the analysis of intracellular staining was performed by flow cytometry. WD-T cells were enumerated and re-stimulated with aAPC at a ratio of 1:1 and supplemented with exogenous cytokines as described for stimulations in section 6.3.

6.9.2 nCounter System Digital Gene Expression Analysis

The day 0 CAR and mIL15-CAR T cells, as well as WD-mIL15-CAR T cells persisting at day 65 were phenotyped and pellets of > 5 x 10^5 cells were frozen at -80˚C for later gene expression analysis by the nCounter System (NanoString Technologies). Gene expression

was analyzed for mIL15-CAR and CAR T cells obtained from cultures after more than 8 days after the fourth aAPC stimulation. The WD-mIL15-CAR T cells were those persisting long-term (>65 days after antigen withdrawal) in cultures from the antigen and exogenous cytokine withdrawal assays. Cells used for analysis had been pelleted and frozen at -80˚C. At the time of analysis, RLT buffer (Qiagen) was added to samples prior to thawing at a ratio of 166ul of buffer per 1 x 10^6 cells. RNA lysates were immediately processed and analyzed using nCounter Analysis System (NanoString Technologies) to detect genes of interest (233) using a lymphocyte-specific CodeSet (**Appendix 1**). This CodeSet was generated from RefSeq accession numbers for chosen mRNA transcripts that were used to generate the reporter and capture probe pairs constituting the lymphocyte gene panel. Multiplexed target-specific color-coded reporter and biotinylated capture probes for mRNA species of interest were hybridized for >12 hours at 65˚C in a thermocycler (Peltier Thermal Cycler, Bio-Rad), processed in the cCounter PrepStation (NanoString Technologies), and then analyzed using the nCounter Digital Analyzer (NanoString Technologies). Reference genes (ACTB, G6PD, OAZ1, POLR1B, POLR2A, RPL27, RPS13, TBP), which together span a dynamic range of RNA expression, were included in the CodeSet and were used to normalize transcript levels to account for variation of transcript levels between samples and in the amount of total RNA present in samples. The count data was positive-, negative-, and housekeeping gene-normalized per nCounter guidelines using the nCounter RCC Collector Worksheet (v1.6.0, NanoString Technologies) as previously described (234) and filtered to remove genes with low counts of less than 10 in greater than 60% of samples. Statistical analysis were performed by edgeR software (235) via RobiNA Java interface (236). Statistical evaluation of differential gene expression was performed using edgeR with implemented statistical methods based on a negative binomial dispersion (235, 237-239). Genes considered to be significantly differentially expressed demonstrated an absolute fold change ≥ 2, a p-value cut-off of 0.01, and a FDR significance value (q) less than 0.03 with the Benjamini-Hochberg method (240) used for multiple testing correction. These significant differentially expressed genes were represented in a heatmap generated using Qlucore Omics Explorer (v.2.3, Qlucore). Gene Ontology classification of significant genes was examined by DAVID functional annotation (http://david.abcc.ncifcrf.gov/)(158, 159).

6.9.3 *In Vivo* Characterization of Persisting mIL15-CAR T Cells

NSG mice were infused with 2 x 10^7 mIL15-CAR T cells (n = 9) that were allowed to engraft at least 50 days. Peripheral blood, spleen, and bone marrow were harvested for analysis by flow cytometry with staining for IL-15, CAR, CD45RA, CD45RO, IL-2Rα, IL-7Ra, CD27, and CCR7.

6.10 Statistical Analysis

We performed statistical analyses using Prism (GraphPad Software, v.6.02). Cell count and flux values were log transformed for statistical analysis. Paired (*in vitro* data) and unpaired (*in vivo* data) t tests were used to compare two groups. One-way ANOVA was used to compare three groups. Two-way ANOVA was used for multiple comparisons of two factors. Two-way RM ANOVA compared multiple groups over time. Survival was analyzed with log-rank (Mantel-Cox).

APPENDICIES

Appendix A. nCounter Analysis System Lymphocyte-specific CodeSet

Gene Name	Accession	Target Region	Target Sequence
ABCB1	NM_000927.3	3910-4010	TATAGCACTAAAGTAGGAGACAAAGGAACTCAGCTCTCTGGTGGCCAGAAACAACGCATTGCC ATAGCTCGTGCCCTTGTTAGACAGCCTCATATTTTGC
ABCG2	NM_004827.2	285-385	AGGATTTAGGAACGCACCGTGCACATGCTTGGTGGTCTTGTTAAGTGGAAACTGCTGCTTTAG AGTTTGTTTGGAAGGTCCGGGTGACTCATCCCAACAT
ACTB	NM_001101.2	1010-1110	TGCAGAAGGAGATCACTGCCCTGGCACCCAGCACAATGAAGATCAAGATCATTGCTCCTCCTG AGCGCAAGTACTCCGTGTGGATCGGCGGCTCCATCCT
ADAM19	NM_023038.3	1690-1790	GAGAAGGTGAATGTGGCAGGAGACACCTTTGGAAACTGTGGAAAGGACATGAATGGTGAACA CAGGAAGTGCAACATGAGAGATGCGAAGTGTGGGAAGA
AGER	NM_001136.3	340-440	GAAAGGAGACCAAGTCCAACTACCGAGTCCGTGTCTACCAGATTCCTGGGAAGCCAGAAATTG TAGATTCTGCCTCTGAACTCACGGCTGGTGTTCCCAA
AHNAK	NM_001620.1	15420-15520	GGATTTGACCTGAATGTTCCTGGGGGTGAAATTGATGCCAGCCTCAAGGCTCCGGATGTAGAT GTCAACATCGCAGGGCCGGATGCTGCACTCAAAGTCG
AIF1	NM_032955.1	315-415	AAAAGCGAGAGAAAAGGAAAAGCCAACAGGCCCCCCAGCCAAGAAAGCTATCTCTGAGTTGC CCTGATTTGAAGGGAAAAGGGATGATGGGATTGAAGGG
AIM2	NM_004833.1	607-707	ACGTGCTGCACCAAAAGTCTCTCCTCATGTTAAGCCTGAACAGAAACAGATGGTGGCCCAGCA GGAATCTATCAGAGAAGGGTTTCAGAAGCGCTGTTTG
AIMP2	NM_006303.3	507-607	CCCTCTCCCTGCTTGTGCTGCACAGGCTGCTCTGTGAGCACTTCAGGGTCCTGTCCACGGTG CACACGCACTCCTCGGTCAAGAGCGTGCCTGAAAACCT
AKIP1	NM_020642.3	570-670	GAACATCTCTAAGGACCTCTACATAGAAGTATATCCAGGGACCTATTCTGTCACTGTGGGCTCA AATGACTTAACCAAGAAGACTCATGTGGTAGCAGTT
AKT1	NM_005163.2	1772-1872	TTCTTTGCCGGTATCGTGTGGCAGCACGTGTACGAGAAGAAGCTCAGCCCACCCTTCAAGCC CCAGGTCACGTCGGAGACTGACACCAGGTATTTTGATG
ALDH1A1	NM_000689.3	11-111	ATTGCTGAGCCAGTCACCTGTGTTCCAGGAGCCGAATCAGAAATGTCATCCTCAGGCACGCCA GACTTACCTGTCCTACTCACCGATTTGAAGATTCAAT
ANXA1	NM_000700.1	515-615	GAAATCAGAGACATTAACAGGGTCTACAGAGAGGAACTGAAGAGAGATCTGGCCAAAGACATA ACCTCAGACACATCTGGAGATTTTCGGAACGCTTTGC
ANXA2P2	NR_003573.1	257-357	ATATTGTCTTCTCCTACCAGAGAAGGACCAAAAAGGAACTTGCATCAGCACTGAAGTCAGCCTT ATCTGGCCACCTGGAGACGGTGATTTTGGGCCTATT
APAF1	NM_181869.1	1160-1260	TTCTGATGAAACTGCAGAATCTTTGCACACGGTTGGATCAGGATGAGAGTTTTTCCCAGAGGC TTCCACTTAATATTGAAGAGGCTAAAGACCGTCTCCG
ARG1	NM_000045.2	505-605	AAGGAACTAAAAGGAAAGATTCCCGATGTGCCAGGATTCTCCTGGGTGACTCCCTGTATATCT GCCAAGGATATTGTGTATATTGGCTTGAGAGACGTGG
ARRB2	NM_004313.3	1652-1752	CATTAATTTTTTGACTGCAGCTCTGCTTCTCCAGCCCCGCCGTGGGTGGCAAGCTGTGTTCAT ACCTAAATTTTCTGGAAGGGGACAGTGAAAAGAGGAG
ATF3	NM_001030287.2	600-700	GGCTCAGAATGGGAGGACTCCAGAAGATGAGAGAAACCTCTTTATCCAACAGATAAAAGAAGG AACATTGCAGAGCTAAGCAGTCGTGGTATGGGGGCGA
ATM	NM_000051.3	30-130	ACGCTAAGTCGCTGGCCATTGGTGGACATGGCGCAGGCGCGTTTGCTCCGACGGGCCGAAT GTTTTGGGGCAGTGTTTTGAGCGCGGAGACCGCGTGATA
ATP2B4	NM_001684.3	7640-7740	CTTCCCATAGTATCATCTGTCCTCTGGAATGACTCTCCTGTCCCTAAAGGGGTTAAGAGAGAG ATCACCTAGAAATCCCTCTGGACACTTGTGGGTTCTT
AXIN2	NM_004655.3	1035-1135	CTTGTCCAGCAAAACTCTGAGGGCCACGGCGAGTGTGAGGTCCACGGAAACTGTTGACAGTG GATACAGGTCCTTCAAGAGGAGCGATCCTGTTAATCCT
B2M	NM_004048.2	25-125	CGGGCATTCCTGAAGCTGACAGCATTCGGGCCGAGATGTCTCGCTCCGTGGCCTTAGCTGTG CTCGCGCTACTCTCTCTTTCTGGCCTGGAGGCTATCCA
B3GAT1	NM_018644.3	145-245	CTGGACAGCGACCCCTTCTCAGACTCCAGTTGGGCCGGACTCTCCAAACCTGCTTCCGCAAT GGGTGGGTTGTGAGTGCTGGTAATGAGGAGCCGTGGGT
BACH2	NM_021813.2	3395-3495	TGTGGCACTGTTCATCTGCTGTCCCGAAGAAACCGAGAACACATTTGGTGCACACTACAGCGG TCTTAGCAGCAATACTGTTCCGAAGTATCCTCTCCTC
BAD	NM_004322.2	195-295	CAGCTGTGCCTTGACTACGTAACATCTTGTCCTCACAGCCCAGAGCATGTTCCAGATCCCAGA GTTTGAGCCGAGTGAGCAGGAAGACTCCAGCTCTGCA
BAG1	NM_004323.3	1490-1590	CTCTTGTGATCGTGTAGTCCCATAGCTGTAAAACCAGAATCACCAGGAGGTTGCACCTAGTCA GGAATATTGGGAATGGCCTAGAACAAGGTGTTTGGCA
BATF	NM_006399.3	825-925	CACTGTGGGTTGCAGGCCCAATGCAGAAGAGTATTAAGAAAGATGCTCAAGTCCCATGGCACA GAGCAAGGCGGGCAGGGAACGGTTATTTTTCTAAATA
BAX	NM_138761.2	694-794	ATTTTTCTGGGAGGGGTGGGGATTGGGGGACATGGGCATTTTTCTTACTTTTGTAATTATTGG GGGGTGTGGGGAAGAGTGGTCTTGAGGGGGTAATAAA
BCL10	NM_003921.2	1250-1350	TGAAAATACCATCTTCTCTTCAACTACACTTCCCAGACCTGGGGACCCAGGGGCTCCTCCTTT GCCACCAGATCTACAGTTAGAAGAAGAAGGAACTTGT
BCL11B	NM_022898.1	3420-3520	GAGATGTAGCACTCATGTCGTCCCGAGTCAAGCGGCCTTTTCTGTGTTGATTTCGGCTTTCATA TTACATAAGGGAAACCTTGAGTGGTGGTGCTGGGGG
BCL2	NM_000633.2	1525-1625	CCAAGCACCGCTTCGTGTGGCTCCACCTGGATGTTCTGTGCCTGTAAACATAGATTCGCTTTC CATGTTGTTGGCCGGATCACCATCTGAAGAGCAGACG

BCL2L1	NM_138578.1	1560-1660	CTAAGAGCCATTTAGGGGCCACTTTTGACTAGGGATTCAGGCTGCTTGGGATAAAGATGCAAG GACCAGGACTCCCTCCTCACCTCTGGACTGGCTAGAG
BCL2L1	NM_001191.2	260-360	ATCTTGGCTTTGGATCTTAGAAGAGAATCACTAACCAGAGACGAGACTCAGTGAGTGAGCAGG TGTTTTGGACAATGGACTGGTTGAGCCCATCCCTATT
BCL2L11	NM_138621.2	2825-2925	TGTTGGCACCAGAACTTAAAGCGATGACTGGATGTCTCTGTACTGTATGTATCTGGTTATCAAG ATGCCTCTGTGCAGAAAGTATGCCTCCCGTGGGTAT
BCL2L11	NM_138621.4	257-357	CGGACTGAGAAACGCAAGAAAAAAAGACCAAATGGCAAAGCAACCTTCTGATGTAAGTTCTGA GTGTGACCGAGAAGGTAGACAATTGCAGCCTGCGGAG
BCL6	NM_001706.2	675-775	GTTGTGGACACTTGCCGGAAGTTTATTAAGGCCAGTGAAGCAGAGATGGTTTCTGCCATCAAG CCTCCTCGTGAAGAGTTCCTCAACAGCCGGATGCTGA
BCL6B	NM_181844.3	2135-2235	CTTTATTTGTTCTAGGGCAGCTCTGGGAACATGCGGGATTGTGGAATTGGGTCAGGAACCCTC TCTGGTATTCTGGATGTTGTAGGTTCTCTAGCAGTCT
BHLHE41	NM_030762.2	655-755	CGCCCATTCAGTCCGACTTGGATGCGTTCCACTCGGGATTTCAAACATGCGCCAAAGAAGTCT TGCAATACCTCTCCCGGTTTGAGAGCTGGACACCCAG
BID	NM_197966.1	2095-2195	GCTTAGCTTTAGAAACAGTGCAACACTGGTCTGCTGTTCCAGTGGTAAGCTATGTCCCAGGAA TCAGTTTAAAAGCACGACAGTGGATGCTGGGTCCATA
BIRC2	NM_001166.3	1760-1860	TGGGATCCACCTCTAAGAATACGTCTCCAATGAGAAACAGTTTTGCACATTCATTATCTCCCAC CTTGGAACATAGTAGCTTGTTCAGTGGTTCTTACTC
BLK	NM_001715.2	990-1090	AGCTTCTTGCTCCAATCAACAAGGCCGGCTCCTTTCTTATCAGAGAGAGTGAAACCAACAAAG GTGCCTTCTCCCTGTCTGTGAAGGATGTCACCACCCA
BMI1	NM_005180.5	1145-1245	CCTGGAGAAGGAATGGTCCACTTCCATTGAAATACAGAGTTCGACCTACTTGTAAAAGAATGA AGATCAGTCACCAGAGAGATGGACTGACAAATGCTGG
BNIP3	NM_004052.2	325-425	CACCTCGCTCGCAGACACCACAAGATACCAACAGGGCTTCTGAAACAGATACCCATAGCATTG GAGAGAAAAACAGCTCACAGTCTGAGGAAGATGATAT
BTLA	NM_001085357.1	890-990	GCACCAACAGAATATGCATCCATATGTGTGAGGAGTTAAGTCTGTTTCTGACTCCAACAGGGA CCATTGAATGATCAGCATGTTGACATCATTGTCTGGG
C21orf33	NM_004649.5	1340-1440	TTGAGTTAATCAGCGTAAGGGGATTTCTAAAGCAGGCAATCCCTGTAGCCGCAGAGAATAAAC GCCTTCCCAAAATGGCAACTTCCCACAGCCACATTTC
CA2	NM_000067.2	575-675	AGCTGTGCAGCAACCTGATGGACTGGCCGTTCTAGGTATTTTTTTGAAGGTTGGCAGCGCTAA ACCGGGCCTTCAGAAAGTTGTTGATGTGCTGGATTCC
CA9	NM_001216.2	960-1060	CAGGTCCCAGGACTGGACATATCTGCACTCCTGCCCTCTGACTTCAGCCGCTACTTCCAATAT GAGGGGTCTCTGACTACACCGCCCTGTGCCCAGGGTG
CARD9	NM_052813.2	1850-1950	CGCTGACTTGGCCTGGAACGAGGAATCTGGTGCCCTGAAAGGCCCAGCCGGACTGCCGGGC ATTGGGGCCGTTTGTTAAGCGGCACTCATTTTGCGGAGG
CASP1	NM_033292.2	575-675	ACAGGCATGACAATGCTGCTACAAAATCTGGGGTACAGCGTAGATGTGAAAAAAAATCTCACT GCTTCGGACATGACTACAGAGCTGGAGGCATTTGCAC
CAT	NM_001752.2	1130-1230	ATGCTTCAGGGCCGCCTTTTTGCCTATCCTGACACTCACCGCCATCGCCTGGGACCCAATTAT CTTCATATACCTGTGAACTGTCCCTACCGTGCTCGAG
CBLB	NM_170662.3	3195-3295	TAATGTCGAAGTTGCCCGGAGCATCCTCCGAGAATTTGCCTTCCCTCCTCCAGTATCCCCACG TCTAAATCTATAGCAGCCAGAACTGTAGACACCAAAA
CCBP2	NM_001296.3	1345-1445	GAACAGATGGGAACCAGCTCAATTGGGTGTCCACTCAAAGTGCTCTCTCCAGGGGCCTCAGT GACTGTGTTGCTAAACCCAGTGGTCAGTTCTCAGTTCT
CCL3	NM_002983.2	681-781	CTGTGTAGGCAGTCATGGCACCAAAGCCACCAGACTGACAAATGTGTATCGGATGCTTTTGTT CAGGGCTGTGATCGGCCTGGGGAAATAATAAAGATGC
CCL4	NM_002984.2	35-135	TTCTGCAGCCTCACCTCTGAGAAAACCTCTTTGCCACCAATACCATGAAGCTCTGCGTGACTG TCCTGTCTCTCCTCATGCTAGTAGCTGCCTTCTGCTC
CCL5	NM_002985.2	280-380	AGTGTGTGCCAACCCAGAGAAGAAATGGGTTCGGGAGTACATCAACTCTTTGGAGATGAGCTA GGATGGAGAGTCCTTGAACCTGAACTTACACAAATTT
CCNB1	NM_031966.2	715-815	AACTTGAGGAAGAGCAAGCAGTCAGACCAAAATACCTACTGGGTCGGGAAGTCACTGGAAAC ATGAGAGCCATCCTAATTGACTGGCTAGTACAGGTTCA
CCND1	NM_053056.2	690-790	TTGAACACTTCCTCTCCAAAATGCCAGAGGCGGAGGAGAACAAACAGATCATCCGCAAACACG CGCAGACCTTCGTTGCCCTCTGTGCCACAGATGTGAA
CCR1	NM_001295.2	535-635	CATCATTTGGGCCCTGGCCATCTTGGCTTCCATGCCAGGCTTATACTTTTCCAAGACCCAATG GGAATTCACTCACCACACCTGCAGCCTTCACTTTCCT
CCR2	NM_001123041.2	20-120	ACATTCTGTTGTGCTCATATCATGCAAATTATCACTAGTAGGAGAGCAGAGAGTGGAAATGTTC CAGGTATAAAGACCCACAAGATAAAGAAGCTCAGAG
CCR4	NM_005508.4	35-135	GGTCCTTCTTAGCATCGTGCTTCCTGAGCAAGCCTGGCATTGCCTCACAGACCTTCCTCAGAG CCGCTTTCAGAAAAGCAAGCTGCTTCTGGTTGGGCCC
CCR5	NM_000579.1	2730-2830	TAGGAACATACTTCAGCTCACACATGAGATCTAGGTGAGGATTGATTACCTAGTAGTCATTTCA TGGGTTGTTGGGAGGATTCTATGAGGCAACCACAGG
CCR6	NM_031409.2	935-1035	CTTTAACTGCGGGATGCTGCTCCTGACTTGCATTAGCATGGACCGGTACATCGCCATTGTACA GGCGACTAAGTCATTCCGGCTCCGATCCAGAACACTA
CCR7	NM_001838.2	1610-1710	TTCCGAAAACCAGGCCTTATCTCCAAGACCAGAGATAGTGGGGAGACTTCTTGGCTTGGTGAG GAAAAGCGGACATCAGCTGGTCAAACAAACTCTCTGA
CD160	NM_007053.2	500-600	TTGATGTTCACCATAAGCCAAGTCACACCGTTGCACAGTGGGACCTACCAGTGTTGTGCCAGA AGCCAGAAGTCAGGTATCCGCCTTCAGGGCCATTTTT
CD19	NM_001770.4	1770-1870	AGATTCACACCTGACTCTGAAATCTGAAGACCTCGAGCAGATGATGCCAACCTCTGGAGCAAT GTTGCTTAGGATGTGTGCATGTGTGTAAGTGTGTGTG
CD19R-scfv	SCFV013.1	204-304	GGCACCGACTACAGCCTGACCATCTCCAACCTGGAGCAGGAGGACATCGCCACCTACTTTTG CCAGCAGGGCAACACACTGCCCTACACCTTTGGCGGCG

CD19RCD28	MDA_00002.1	2-102	CAGGTGTTCCTGAAGATGAACAGCCTGCAGACCGACGACACCGCCATCTACTACTGTGCCAA GCACTACTACTACGGCGGCAGCTACGCCATGGACTACT
CD2	NM_001767.2	1400-1500	TGGGTCTCACTACAAGCAGCCTATCTGCTTAAGAGACTCTGGAGTTTCTTATGTGCCCTGGTG GACACTTGCCCACCATCCTGTGAGTAAAAGTGAAATA
CD20-scfv (rutuximab)	SCFV002.1	8-108	GCTGTCCCAGAGCCCCGCCATCCTGAGCGCCAGCCCTGGCGAGAAGGTGACCATGACCTGC CGGGCCAGCAGCTCTGTGAGCTACATGCACTGGTATCAG
CD226	NM_006566.2	163-263	TAAACAGGATACGATAAAAGTCCTTAACCAAGACGCAGATGGGAAGAAGCGTTAGAGCGAGCA GCACTCACATCTCAAGAACCAGCCTTTCAAACAGTTT
CD244	NM_016382.2	1150-1250	AAGAGGAACCACAGCCCTTCCTTCAATAGCACTATCTATGAAGTGATTGGAAAGAGTCAACCTA AAGCCCAGAACCCTGCTCGATTGAGCCGCAAAGAGC
CD247	NM_198053.1	1490-1590	TGGCAGGACAGGAAAAACCCGTCAATGTACTAGGATACTGCTGCGTCATTACAGGGCACAGG CCATGGATGGAAAACGCTCTCTGCTCTGCTTTTTTTCT
CD27	NM_001242.4	330-430	CCAGATGTGTGAGCCAGGAACATTCCTCGTGAAGGACTGTGACCAGCATAGAAAGGCTGCTC AGTGTGATCCTTGCATACCGGGGGTCTCCTTCTCTCCT
CD274	NM_014143.2	684-784	TAGGAGATTAGATCCTGAGGAAAACCATACAGCTGAATTGGTCATCCCAGAACTACCTCTGGC ACATCCTCCAAATGAAAGGACTCACTTGGTAATTCTG
CD276	NM_001024736.1	2120-2220	ACATTTCTTAGGGACACAGTACACTGACCACATCACCACCCTCTTCTTCCAGTGCTGCGTGGA CCATCTGGCTGCCTTTTTTCTCCAAAAGATGCAATAT
CD28	NM_006139.1	305-405	GCTTGTAGCGTACGACAATGCGGTCAACCTTAGCTGCAAGTATTCCTACAATCTCTTCTCAAGG GAGTTCCGGGCATCCCTTCACAAAGGACTGGATAGT
CD300A	NM_007261.2	0-100	CGGGGAAGTGAGAGTCGGGGATCAGTCCTGCAAGCTACGGAGTCACTACAGGGAGAGGTCT CATCACTAGAAATAGCCGAAGAACCTGCAGCCTCAACCA
CD38	NM_001775.2	1035-1135	CCTTGACTCCTTGTGGTTTATGTCATCATACATGACTCAGCATACCTGCTGGTGCAGAGCTGAA GATTTTGGAGGGTCCTCCACAATAAGGTCAATGCCA
CD3D	NM_000732.4	110-210	TATCTACTGGATGAGTTCCGCTGGGAGATGGAACATAGCACGTTTCTCTCTGGCCTGGTACTG GCTACCCTTCTCTCGCAAGTGAGCCCCTTCAAGATAC
CD3E	NM_000733.2	75-175	AAGTAACAGTCCCATGAAACAAAGATGCAGTCGGGCACTCACTGGAGAGTTCTGGGCCTCTG CCTCTTATCAGTTGGCGTTTGGGGGCAAGATGGTAATG
CD4	NM_000616.3	835-935	AGACATCGTGGTGCTAGCTTTCCAGAAGGCCTCCAGCATAGTCTATAAGAAAGAGGGGGAAC AGGTGGAGTTCTCCTTCCCACTCGCCTTTACAGTTGAA
CD40LG	NM_000074.2	1225-1325	GCATTTGATTTATCAGTGAAGATGCAGAAGGGAAATGGGGAGCCTCAGCTCACATTCAGTTAT GGTTGACTCTGGGTTCCTATGGCCTTGTTGGAGGGGG
CD44	NM_000610.3	2460-2560	GTGGGCAGAAGAAAAAGCTAGTGATCAACAGTGGCAATGGAGCTGTGGAGGACAGAAAGCCA AGTGGACTCAACGGAGAGGCCAGCAAGTCTCAGGAAAT
CD45R-scfv	SCFV006.1	222-322	TTCACCCTGAACATCCACCCCGTGGAGGAAGAGGACGCCGCCACCTACTACTGCCAGCACAG CAGAGAGCTGCCCTTCACCTTCGGCTCCGGCACCAAGC
CD47	NM_001777.3	897-997	GCCATATTGGTTATTCAGGTGATAGCCTATATCCTCGCTGTGGTTGGACTGAGTCTCTGTATTG CGGCGTGTATACCAATGCATGGCCCTCTTCTGATTT
CD56R-scfv	SCFV008.1	197-297	ATTCAGCGGCTCTGGCTCCGGCACCGACTTCACTCTGATGATCTCTCGGGTGGAGGCCGAGG ACCTGGGCGTGTACTACTGCTTTCAGGGCAGCCACGTG
CD58	NM_001779.2	478-578	GTGCTTGAGTCTCTTCCATCTCCCACACTAACTTGTGCATTGACTAATGGAAGCATTGAAGTCC AATGCATGATACCAGAGCATTACAACAGCCATCGAG
CD63	NM_001780.4	350-450	GTCATCATCGCAGTGGGTGTCTTCCTCTTCCTGGTGGCTTTTGTGGGCTGCTGCGGGGCCTG CAAGGAGAACTATTGTCTTATGATCACGTTTGCCATCT
CD69	NM_001781.1	460-560	AGGACATGAACTTTCTAAAACGATACGCAGGTAGAGAGGAACACTGGGTTGGACTGAAAAAGG AACCTGGTCACCCATGGAAGTGGTCAAATGGCAAAGA
CD7	NM_006137.6	440-540	CCTACACCTGCCAGGCCATCACGGAGGTCAATGTCTACGGCTCCGGCACCCTGGTCCTGGTG ACAGAGGAACAGTCCCAAGGATGGCACAGATGCTCGGA
CD80	NM_005191.3	1288-1388	AAAGATCTGAAGGTCCCACCTCCATTTGCAATTGACCTCTTCTGGGAACTTCCTCAGATGGACA AGATTACCCCACCTTGCCCTTTACGTATCTGCTCTT
CD86	NM_006889.3	146-246	TATGGGACTGAGTAACATTCTCTTTGTGATGGCCTTCCTGCTCTCTGGTGCTGCTCCTCTGAAG ATTCAAGCTTATTTCAATGAGACTGCAGACCTGCCA
CD8A	NM_001768.5	1320-1420	GCTCAGGGCTCTTTCCTCCACACCATTCAGGTCTTTCTTTCCGAGGCCCCTGTCTCAGGGTGA GGTGCTTGAGTCTCCAACGGCAAGGGAACAAGTACTT
CDH1	NM_004360.2	1230-1330	CGATAATCCTCCGATCTTCAATCCCACCACGTACAAGGGTCAGGTGCCTGAGAACGAGGCTAA CGTCGTAATCACCACACTGAAAGTGACTGATGCTGAT
CDK2	NM_001798.2	220-320	TCGCTGGCGCTTCATGGAGAACTTCCAAAAGGTGGAAAAGATCGGAGAGGGCACGTACGGAG TTGTGTACAAAGCCAGAAACAAGTTGACGGGAGAGGTG
CDK4	NM_000075.2	1055-1155	ACTTTTAACCCACACAAGCGAATCTCTGCCTTTCGAGCTCTGCAGCACTCTTATCTACATAAGG ATGAAGGTAATCCGGAGTGAGCAATGGAGTGGCTGC
CDKN1A	NM_000389.2	1975-2075	CATGTGTCCTGGTTCCCGTTTCTCCACCTAGACTGTAAACCTCTCGAGGGCAGGGACCACACC CTGTACTGTTCTGTGTCTTTCACAGCTCCTCCCACAA
CDKN1B	NM_004064.2	365-465	GCTTCCGAGAGGGGTTCGGGCCGCGTAGGGGCGCTTTGTTTTGTTCGGTTTTGTTTTTTTGAG AGTGCGAGAGAGGCGGTCGTGCAGACCCGGGAGAAAG
CDKN2A	NM_000077.3	975-1075	AAGCGCACATTCATGTGGGCATTTCTTGCGAGCCTCGCAGCCTCCGGAAGCTGTCGACTTCAT GACAAGCATTTTGTGAACTAGGGAAGCTCAGGGGGGT
CDKN2C	NM_001262.2	1295-1395	ATAATGTAAACGTCAATGCACAAAATGGATTTGGAAGGACTGCGCTGCAGGTTATGAAACTTG GAAATCCCGAGATTGCCAGGAGACTGCTACTTAGAGG
CEBPA	NM_004364.2	1320-1420	GAGCTGGGAGCCCGGCAACTCTAGTATTTAGGATAACCTTGTGCCTTGGAAATGCAAACTCAC CGCTCCAATGCCTACTGAGTAGGGGGAGCAAATCGTG

CFLAR	NM_003879.3	445-545	CAAGACCCTTGTGAGCTTCCCTAGTCTAAGAGTAGGATGTCTGCTGAAGTCATCCATCAGGTT GAAGAAGCACTTGATACAGATGAGAAGGAGATGCTGC
CFLAR	NM_001127183.1	653-753	TAGAGTGCTGATGGCAGAGATTGGTGAGGATTTGGATAAATCTGATGTGTCCTCATTAATTTTC CTCATGAAGGATTACATGGGCCGAGGCAAGATAAGC
CHPT1	NM_020244.2	1303-1403	GATATGGTGATATACTTTAGTGCTTTGTGCCTGCAAATTTCAAGACACCTTCATCTAAATATATT CAAGACTGCATGTCATCAAGCACCTGAACAGGTTC
CIITA	NM_000246.3	470-570	GCCTGAGCAAGGACATTTTCAAGCACATAGGACCAGATGAAGTGATCGGTGAGAGTATGGAG ATGCCAGCAGAAGTTGGGCAGAAAAGTCAGAAAAGACC
CITED2	NM_006079.3	965-1065	AGGAGCTGCCCGAACTCTGGCTGGGGCAAAACGAGTTTGATTTTATGACGGACTTCGTGTGC AAACAGCAGCCCAGCAGAGTGAGCTGTTGACTCGATCG
CLIC1	NM_001288.4	310-410	GTGATGGGGCCAAGATTGGGAACTGCCCATTCTCCCAGAGACTGTTCATGGTACTGTGGCTCA AGGGAGTCACCTTCAATGTTACCACCGTTGACACCAA
CLNK	NM_052964.2	1108-1208	GAAGGAGAACAAGGATGGTAGTTTCTTGGTCCGAGATTGTTCCACAAAATCCAAGGAAGAGCC CTATGTTTTGGCTGTGTTTTATGAGAACAAAGTCTAC
c-MET-scfv	SCFV004.1	138-238	CTGATCTACGCCGCCAGCAGCCTGAAGAGCGGCGTGCCCAGCCGGTTTAGCGGCTCTGGCT CTGGCGCCGACTTCACCCTGACCATCAGCAGCCTGCAGC
CREB1	NM_004379.3	4855-4955	TTTGATGGTAGGTCAGCAGCAGTGCTAGTCTCTGAAAGCACAATACCAGTCAGGCAGCCTATC CCATCAGATGTCATCTGGCTGAAGTTTATCTCTGTCT
CREM	NM_001881.2	260-360	CTCCACCTCCTCGCGTCCGTAATCAGTGACGAGGTCCGCTACGTAAATCCCTTTGCGGCGGA CAAATGACCATGGAAACAGTTGAATCCCAGCATGATGG
CRIP1	NM_001311.4	269-369	CAACCACCCCTGCTACGCAGCCATGTTTGGGCCTAAAGGCTTTGGGCGGGGCGGAGCCGAG AGCCACACTTTCAAGTAAACCAGGTGGTGGAGACCCCAT
CRLF2	NM_022148.2	1420-1520	CAAGGCAGCACGTCCAAAATGCTGTAAAACCATCTTCCCACTCTGTGAGTCCCCAGTTCCGTC CATGTACCTGTTCCATAGCATTGGATTCTCGGAGGAT
CSAD	NM_015989.4	205-305	TCAAATTCTTCTGCCTAGCCTTAGCCATTAGAGAGAGGTCCTGCTAAAGATGGACTGCAAATG CGCTTGATGGAAGGAGATGTCAATTCCACTGAAGTCC
CSF2	NM_000758.2	475-575	AGATGAGGCTGGCCAAGCCGGGGAGCTGCTCTCTCATGAAACAAGAGCTAGAAACTCAGGAT GGTCATCTTGGAGGGACCAAGGGGTGGGCCACAGCCAT
CSNK2A1	NM_177559.2	1930-2030	CCATTCCCACCATTGTTCCTCCACCGTCCCACACTTTAGGGGGTTGGTATCTCGTGCTCTTCTC CAGAGATTACAAAAATGTAGCTTCTCAGGGGAGGCA
CTGF	NM_001901.2	1100-1200	ACCACCCTGCCGGTGGAGTTCAAGTGCCCTGACGGCGAGGTCATGAAGAAGAACATGATGTT CATCAAGACCTGTGCCTGCCATTACAACTGTCCCGGAG
CTLA4	NM_005214.3	405-505	AGTCTGTGCGGCAACCTACATGATGGGGAATGAGTTGACCTTCCTAGATGATTCCATCTGCAC GGGCACCTCCAGTGGAAATCAAGTGAACCTCACTATC
CTNNA1	NM_001903.2	75-175	TCGCCCAGCTAGCCGCAGAAATGACTGCTGTCCATGCAGGCAACATAAACTTCAAGTGGGATC CTAAAAGTCTAGAGATCAGGACTCTGGCAGTTGAGAG
CTNNB1	NM_001098210.1	1815-1915	TCTTGCCCTTTGTCCCGCAAATCATGCACCTTTGCGTGAGCAGGGTGCCATTCCACGACTAGT TCAGTTGCTTGTTCGTGCACATCAGGATACCCAGCGC
CTNNBL1	NM_030877.3	855-955	TGATGCCAACAAACTGTATTGCAGTGAAGTGCTGGCCATATTGCTCCAGGACAATGATGAAAA CAGGGAATTGCTTGGGGAGCTGGATGGAATCGATGTG
CTSC	NM_001114173.1	260-360	TGCTCGGTTATGGGACCACAAGAAAAAAAAGTAGTGGTGTACCTTCAGAAGCTGGATACAGCA TATGATGACCTTGGCAATTCTGGCCATTTCACCATCA
CTSD	NM_001909.3	1495-1595	GAAGCCGGCGGCCCAAGCCCGACTTGCTGTTTTGTTCTGTGGTTTTCCCCTCCCTGGGTTCAG AAATGCTGCCTGCCTGTCTGTCTCTCCATCTGTTTGG
CX3CL1	NM_002996.3	140-240	AGCACCACGGTGTGACGAAATGCAACATCACGTGCAGCAAGATGACATCAAAGATACCTGTAG CTTTGCTCATCCACTATCAACAGAACCAGGCATCATG
CX3CR1	NM_001337.3	1040-1140	GGGCGCTCAGTCCACGTTGATTTCTCCTCATCTGAATCACAAAGGAGCAGGCATGGAAGTGTT CTGAGCAGCAATTTTACTTACCACACGAGTGATGGAG
CXCL10	NM_001565.1	40-140	GCAGAGGAACCTCCAGTCTCAGCACCATGAATCAAACTGCGATTCTGATTTGCTGCCTTATCTT TCTGACTCTAAGTGGCATTCAAGGAGTACCTCTCTC
CXCL12	NM_199168.2	505-605	GGGCCTGAGGTTTGCCAGCATTTAGACCCTGCATTTATAGCATACGGTATGATATTGCAGCTTA TATTCATCCATGCCCTGTACCTGTGCACGTTGGAAC
CXCL9	NM_002416.1	1975-2075	CACCATCTCCCATGAAGAAAGGGAACGGTGAAGTACTAAGCGCTAGAGGAAGCAGCCAAGTC GGTTAGTGGAAGCATGATTGGTGCCCAGTTAGCCTCTG
CXCR1	NM_000634.2	1950-2050	GCAGCCACCAGTCCATTGGGCAGGCAGATGTTCCTAATAAAGCTTCTGTTCCGTGCTTGTCCC TGTGGAAGTATCTTGGTTGTGACAGAGTCAAGGGTGT
CXCR3	NM_001504.1	80-180	GTGAGTGACCACCAAGTGCTAAATGACGCCGAGGTTGCCGCCCTCCTGGAGAACTTCAGCTC TTCCTATGACTATGGAGAAAACGAGAGTGACTCGTGCT
CXCR4	NM_001008540.1	135-235	GTCACTATGGGAAAAGATGGGGAGGAGAGTTGTAGGATTCTACATTAATTCTCTTGTGCCCTT AGCCCACTACTTCAGAATTTCCTGAAGAAAGCAAGCC
DAPL1	NM_001017920.2	190-290	CGAGAAAACAAGTGCCATTGCAAATGTTGCCAAAATACAGACACTGGATGCCCTGAATGACGC ACTGGAGAAGCTCAACTATAAATTTCCAGCAACAGTG
DEC1	NM_017418.2	190-290	AGGCCTTACTTTCCAGATCCAGATCCTTGTGCATACAACTGACTTGTGTGGGTGAGGCTTGCA GAAAAAATCAGCTAGAACAGCCTTGGGGGTAGTGGCA
DECTIN-1R	SCFV010.1	270-370	CTGAAGATCGACAGCAGCAACGAGCTGGGCTTCATCGTGAAGCAGGTGTCCAGCCAGCCCGA CAACTCCTTCTGGATCGGCCTGAGCAGGCCCCAGACCG
DGKA	NM_001345.4	1375-1475	TTCCTAACACCCACCCACTTCTCGTCTTTGTCAATCCTAAGAGTGGCGGGAAGCAGGGGCAAA GGGTGCTCTGGAAGTTCCAGTATATATTAAACCCTCG
DOCK5	NM_024940.6	630-730	TGCGAGATGACAATGGGAACATCCTAGACCCTGACGAAACCAGCACCATTGCCCTCTTCAAGG CCCATGAGGTGGCCTCCAAAAGGATTGAGGAAAAGAT

DOK2	NM_003974.2	650-750	GCCAGGGACCCAGCTGTACGACTGGCCCTACAGGTTTCTGCGGCGCTTTGGGCGGGACAAG GTAACCTTTTCCTTTGAGGCAGGCCGTCGCTGCGTCTCT
DPP4	NM_001935.3	2700-2800	CAGCAGTCAGCTCAGATCTCCAAAGCCCTGGTCGATGTTGGAGTGGATTTCCAGGCAATGTG GTATACTGATGAAGACCATGGAATAGCTAGCAGCACAG
DUSP16	NM_030640.2	615-715	ATGGGTTTAACTCTCCTTTTGCCAGTCACCACCAGCCTGACCTCATACACTTTTAGTACAATGG AGTGGCTGAGCCTTTGAGCACACCACCATTACATCA
EGFR-scfv_ (NIMO CAR)	SCFV015.1	7-107	AGATGACCCAGAGCCCTAGCAGCCTGAGCGCCAGCGTGGGCGACAGAGTGACCATCACCTG CCGGTCCAGCCAGAACATCGTGCACAGCAACGGCAACAC
EGLN1	NM_022051.1	3975-4075	AGCAGCATGGACGACCTGATACGCCACTGTAACGGGAAGCTGGGCAGCTACAAAATCAATGG CCGGACGAAAGCCATGGT
EGLN3	NM_022073.3	800-900	AAGCTACATGGTGGGATCCTGCGGATATTTCCAGAGGGGAAATCATTCATAGCAGATGTGGAG CCCATTTTTGACAGACTCCTGTTCTTCTGGTCAGATC
EIF1	NM_005801.3	869-969	CCTGAACAGTCCTCGGTGAATCTGAGAGGAGAGGATGGGGTAAGGCAGAAGCACCAGCTGTA CTACTAGAAGGGAGCTTTTGGTGGTAGATCCCCTGGTG
ELF4	NM_001421.3	335-435	AGCTCTGGAGGGCTCTGATAATCCCGTTGTCAGCTCTCTGAAAAGACAGCATGGCTATTACCC TACAGCCCAGTGACCTGATCTTTGAGTTCGCAAGCAA
ELOF1	NM_032377.3	125-225	AGACCCAGTTCACCTGCCCCTTCTGCAACCACGAGAAATCCTGTGATGTGAAAATGGACCGTG CCCGCAACACCGGAGTCATCTCTTGTACCGTGTGCCT
ENTPD1	NM_001776.4	225-325	TTCGAGTAACTTTAGGAAAATGAGCTGCTGGACTCCTCAGTCAATCTGTCCTTTCTAGTCAATG AAAAAGACAGGGTTTGAGGTTCCTTCCGAAACGGGG
EOMES	NM_005442.2	1670-1770	ATCCCATGCCCTGGGGTATTACCCAGACCCAACCTTTCCTGCAATGGCAGGGTGGGGAGGTC GAGGTTCTTACCAGAGGAAGATGGCAGCTGGACTACCA
EPHA2	NM_004431.2	1525-1625	GAGCCGAGTGTGGAAGTACGAGGTCACTTACCGCAAGAAGGGAGACTCCAACAGCTACAATG TGCGCCGCACCGAGGGTTTCTCCGTGACCCTGGACGAC
EPHA4	NM_004438.3	20-120	GCAGCGTTGGCACCGGCGAACCATGGCTGGGATTTTCTATTTCGCCCTATTTTCGTGTCTCTT CGGGATTTGCGACGCTGTCACAGGTTCCAGGGTATAC
EPHB2	NM_017449.2	785-885	CAAAGCAGGCTTCGAGGCCGTTGAGAATGGCACCGTCTGCCGAGGTTGTCCATCTGGGACTT TCAAGGCCAACCAAGGGGATGAGGCCTGTACCCACTGT
ETV6	NM_001987.4	3840-3940	GTATGAATATGAAATCAGAGACCAGGGCATGATGTTGCTAGGATTAGAGCCTCTCAGTCTGGC CTCTTCACCCAAGTGCAAGAACTCAGTCTCTTACTGT
FADD	NM_003824.2	1560-1660	TGAGACTGCTAAGTAGGGGCAGTGATGGTTGCCAGGACGAATTGAGATAATATCTGTGAGGT GCTGATGAGTGATTGACACACAGCACTCTCTAAATCTT
FAM129A	NM_052966.2	3526-3626	TGCCCAATAGATTCAAGAGAAGCTAAGCGGAAATGGAGGGTGGAAGGTGTGATCTGTGGGAC TGTCTGGGCCTGTTACTCATCCTGCTATCAATTTCTTA
FANCC	NM_000136.2	2130-2230	GACTCAGTCAGACATGTTCACTAATGACTCAAGTGAGCCTTCGGTACTCCTGGTGCCCGCCCG GCCAGACCGTCAGCTTGATAATTACTAAAGCAAAGGC
FAS	NM_000043.3	90-190	CACCGGGGCTTTTCGTGAGCTCGTCTCTGATCTCGCGCAAGAGTGACACACAGGTGTTCAAA GACGCTTCTGGGGAGTGAGGGAAGCGGTTTACGAGTGA
FASLG	NM_000639.1	625-725	TCCATGCCTCTGGAATGGGAAGACACCTATGGAATTGTCCTGCTTTCTGGAGTGAAGTATAAG AAGGGTGGCCTTGTGATCAATGAAACTGGGCTGTACT
FCGR3B	NM_000570.3	73-173	CCTATTCCTGTTCTATGGTGGGGCTCCATTGCGAGACTTCAGATTGAGAAATCAGATGAAGTTT CAAGAAAAGGAAACTGGCAGGTGACAGAGATGGGTG
FGL2	NM_006682.2	250-350	CAATTCAGCAGGATCGAGGAGGTGTTCAAAGAAGTCCAAAACCTCAAGGAAATCGTAAATAGT CTAAAGAAATCTTGCCAAGACTGCAAGCTGCAGGCTG
FLT1	NM_002019.2	5615-5715	TTCAACTGCTTTGAAACTTGCCTGGGGTCTGAGCATGATGGGAATAGGGAGACAGGGTAGGA AAGGGCGCCTACTCTTCAGGGTCTAAAGATCAAGTGGG
FLT3LG	NM_001459.2	927-1027	CCTCCCCAGAATGGAGGCAACGCCAGAATCCAGCACCGGCCCCATTTACCCAACTCTGTACA AAGCCCTTGTCCCCATGAAATTGTATATAAATCATCCT
FOS	NM_005252.2	1475-1575	ACTCAAGTCCTTACCTCTTCCGGAGATGTAGCAAAACGCATGGAGTGTGTATTGTTCCCAGTG ACACTTCAGAGAGCTGGTAGTTAGTAGCATGTTGAGC
FOXO1	NM_002015.3	1526-1626	TCTCATCACCAACATCATTAACTGTTTCGACCCAGTCCTCACCTGGCACCATGATGCAGCAGA CGCCGTGCTACTCGTTTGCGCCACCAAACACCAGTTT
FOXO3	NM_001455.2	1860-1960	CCGGAACGTGATGCTTCGCAATGATCCGATGATGTCCTTTGCTGCCCAGCCTAACCAGGGAA GTTTGGTCAATCAGAACTTGCTCCACCACCAGCACCAA
FOXP1	NM_032682.5	6758-6858	CCTGAAAATCAGATTTACAATGCTGAAGGCATTTCTTGGGCCCAGTGTAGCTCACGCAATCTCT GCTACCCATAAGCCTTGATGAAGATGATACAGTCCG
FOXP3	NM_014009.3	1230-1330	GGGCCATCCTGGAGGCTCCAGAGAAGCAGCGGACACTCAATGAGATCTACCACTGGTTCACA CGCATGTTTGCCTTCTTCAGAAACCATCCTGCCACCTG
FYN	NM_002037.3	765-865	GTCTTTGGAGGTGTGAACTCTTCGTCTCATACGGGGACCTTGCGTACGAGAGGAGGAACAGG AGTGACACTCTTTGTGGCCCTTTATGACTATGAAGCAC
FZD1	NM_003505.1	2430-2530	GTGCCAATCCTGACATCTCGAGGTTTCCTCACTAGACAACTCTCTTTCGCAGGCTCCTTTGAAC AACTCAGCTCCTGCAAAAGCTTCCGTCCCTGAGGCA
G6PD	NM_000402.2	1155-1255	ACAACATCGCCTGCGTTATCCTCACCTTCAAGGAGCCCTTTGGCACTGAGGGTCGCGGGGGC TATTTCGATGAATTTGGGATCATCCGGGACGTGATGCA
GABPA	NM_002040.3	1160-1260	GACCAAGTCCTGCATTGGGTGGTTTGGGTAATGAAGGAATTCAGCATGACCGATATAGACCTC ACCACACTCAACATTTCGGGGAGAGAATTATGTAGTC
GADD45A	NM_001924.2	865-965	GTTACTCCCTACACTGATGCAAGGATTACAGAAACTGATGCCAAGGGGCTGAGTGAGTTCAAC TACATGTTCTGGGGGCCCGGAGATAGATGACTTTGCA
GADD45B	NM_015675.2	365-465	TGTGGACCCAGACAGCGTGGTCCTCTGCCTCTTGGCCATTGACGAGGAGGAGGAGGATGACA TCGCCCTGCAAATCCACTTCACGCTCATCCAGTCCTTC

GAL3ST4	NM_024637.4	1140-1240	CGAGCCCAAACCCTCAATCCCAATGCCCTCATCCATCCTGTTTCCACTGTTACTGATCATCGCA GCCAGATATCAAGCCCTGCCTCTTTCGATTTGGGGT
GAS2	NM_005256.3	915-1015	GATCTCCCGTGTGGATGGCAAAACATCCCCTATCCAAAGCAAATCTCCAACTCTAAAGGACAT GAATCCAGATAACTACTTGGTGGTCTCTGCCAGTTAT
GATA2	NM_032638.3	1495-1595	GAAGAAGGAAGGGATCCAGACTCGGAACCGGAAGATGTCCAACAAGTCCAAGAAGAGCAAGA AAGGGGCGGAGTGCTTCGAGGAGCTGTCAAAGTGCATG
GATA3	NM_001002295.1	2835-2935	AAGAGTCCGGCGGCATCTGTCTTGTCCCTATTCCTGCAGCCTGTGCTGAGGGTAGCAGTGTAT GAGCTACCAGCGTGCATGTCAGCGACCCTGGCCCGAC
gBAD-1R-scfv	SCFV001.1	1-101	AGACAGACACCCTGCTCCTCTGGGTGTCCGGCACCTGTGGCGACATCGTGATGAGCAGAAGC CCCAGCAGCCTGGCCGTGTCCGTGGGCGAGAAAGTGAC
GEMIN2	NM_001009182.1	537-637	ACAAGCAACAGTAACTAGTGTCTTGGAATATCTGAGTAATTGGTTTGGAGAAAGAGACTTTACT CCAGAATTGGGAAGATGGCTTTATGCTTTATTGGCT
GFI1	NM_005263.2	2235-2335	TCATCACTGGAGGTAAAAGCACAAGCAATGCCTGTGGACAAGATGTCATTCATTCACTCAGCA AATGTTCATGGATCACCGGCTACCAAGGTACCAGGCA
GLIPR1	NM_006851.2	255-355	CTGCGTTCGAATCCATAACAAGTTCCGATCAGAGGTGAAACCAACAGCCAGTGATATGCTATA CATGACTTGGGACCCAGCACTAGCCCAAATTGCAAAA
GLO1	NM_006708.1	1240-1340	GGAAATGATATGGTACCCAGACACTGGGCTAGGCTGCAACTTTATCTCATTTAATACTCCCAGC TGTCATGTGAGAAAGAAAGCAGGCTAGGCATGTGAA
GNLY	NM_006433.2	305-405	CAGGAGCTGGGCCGTGACTACAGGACCTGTCTGACGATAGTCCAAAAACTGAAGAAGATGGT GGATAAGCCCACCCAGAGAAGTGTTTCCAATGCTGCGA
GSK3B	NM_002093.2	925-1025	ACTGATTATACCTCTAGTATAGATGTATGGTCTGCTGGCTGTGTGTTGGCTGAGCTGTTACTAG GACAACCAATATTTCCAGGGGATAGTGGTGTGGATC
GZMA	NM_006144.2	155-255	AGACCCTACATGGTCCTACTTAGTCTTGACAGAAAAACCATCTGTGCTGGGGCTTTGATTGCAA AAGACTGGGTGTTGACTGCAGCTCACTGTAACTTGA
GZMB	NM_004131.3	540-640	ACACTACAAGAGGTGAAGATGACAGTGCAGGAAGATCGAAAGTGCGAATCTGACTTACGCCAT TATTACGACAGTACCATTGAGTTGTGCGTGGGGGACC
GZMH	NM_033423.3	705-805	AAAAAAGGGACACCTCCAGGAGTCTACATCAAGGTCTCACACTTCCTGCCCTGGATAAAGAGA ACAATGAAGCGCCTCTAACAGCAGGCATGAGACTAAC
HCST	NM_001007469.1	132-232	ATCCTCTTCCTGCTTTTGCTCCCAGTGGCTGCAGCTCAGACGACTCCAGGAGAGAGATCATCA CTCCCTGCCTTTTACCCTGGCACTTCAGGCTCTTGTT
HDAC1	NM_004964.2	785-885	CAAGCCGGTCATGTCCAAAGTAATGGAGATGTTCCAGCCTAGTGCGGTGGTCTTACAGTGTG GCTCAGACTCCCTATCTGGGGATCGGTTAGGTTGCTTC
HDAC2	NM_001527.1	930-1030	AAGCCTATTATCTCAAAGGTGATGGAGATGTATCAACCTAGTGCTGTGGTATTACAGTGTGGT GCAGACTCATTATCTGGTGATAGACTGGGTTGTTTCA
HER2-scfv	SCFV014.1	64-164	CCTGCAGCGCCAGCAGCAGCGTGTCCTACATGCACTGGTATCAGCAGAAGTCCGGCACTAGC CCCAAGCGGTGGATCTACGACACCTACAAGCTCGCCAG
HERV-K 6H5-scfv	SCFV012.1	137-237	CGGCGGCACCAGCTACAACCAGAAGTTCAAGGACAAGGCCATCCTGACCGTGGACAAGAGCA GCAGCACCGCCTACATGGAACTGCGGAGCCTGACCAGC
HLA-A	NM_002116.5	1000-1100	GGAAGAGCTCAGATAGAAAAGGAGGGAGTTACACTCAGGCTGCAAGCAGTGACAGTGCCCAG GGCTCTGATGTGTCCCTCACAGCTTGTAAAGTGTGAGA
HMGB2	NM_001130688.1	125-225	CTGTCAACATGGGTAAAGGAGACCCCAACAAGCCGCGGGGCAAAATGTCCTCGTACGCCTTC TTCGTGCAGACCTGCCGGGAAGAGCACAAGAAGAAACA
HOPX	NM_001145460.1	1117-1217	AACAATAGGAAGCTATGTGTATCTTCTGTGTAAAGCAGTGGCTTCACTGGAAAAATGGTGTGG CTAGCATTTCCCTTTGAGTCATGATGACAGATGGTGT
HOXA10	NM_018951.3	1503-1603	TTCTATAGAGATAGATATTGTCCTAAGTGTCAAGTCCTGACTGGGCTGGGTTTGCTGTCTTGGG GTCCCACTGCTCGAAATGGCCCCTGTCTTCGGCCGA
HOXA9	NM_152739.3	1015-1115	GGCTCTAAACCTCAGGCCACATCTTTTCCAAGGCAAACCCTGTTCAGGCTGGCTCGTAGGCCT GCCGCTTTGATGGAGGAGGTATTGTAAGCTTTCCATT
HOXB3	NM_002146.4	60-160	TGTCCGTTTAAATGCTGCTGGGAGACTCGTAAAAAAATCATCGTGGACCTGGAGGATGAGAGG GGCGAGCTTTATTTCGGTCGGATTGCGGTGTGGTGGT
HOXB4	NM_024015.4	1340-1440	CCTTTCTTTGTCCCCCACTCCCGATACCCAGCGAAAGCACCCTCTGACTGCCAGATAGTGCAG TGTTTTGGTCACGGTAACACACACACACTCTCCCTCA
HPRT1	NM_000194.1	240-340	TGTGATGAAGGAGATGGGAGGCCATCACATTGTAGCCCTCTGTGTGCTCAAGGGGGGCTATA AATTCTTTGCTGACCTGCTGGATTACATCAAAGCACTG
HRH1	NM_000861.2	3055-3155	GTGGCAGCTCAAAATGATATGTTTGAGTAGACGAACAGCTGACATGGAGTTCCCGTGCACCTA CGGAAGGGGACGCTTTGAAGGAACCAAGTGCATTTTT
HRH2	NM_022304.1	600-700	GCGGTCCTCATCCTCATCACCGTTGCTGGCAATGTGGTCGTCTGTCTGGCCGTGGGCTTGAA CCGCCGGCTCCGCAACCTGACCAATTGTTTCATCGTGT
Human CD19R-scfv	SCFV009.1	215-315	CTTCACCATCAGCAGCCTGCAGCCCGAGGACATCGCCACCTACTACTGCCAGCAGTACCAGA GCCTGCCCTACACCTTCGGCCAGGGCACCAAGCTGCAG
ICOS	NM_012092.2	640-740	AACTCTGGCACCCAGGCATGAAGCACGTTGGCCAGTTTTCCTCAACTTGAAGTGCAAGATTCT CTTATTTCCGGGACCACGGAGAGTCTGACTTAACTAC
ICOSLG	NM_015259.4	1190-1290	CTGCTGGCGTTGGCTGTGATCCTGGAATGAGGCCCTTTCAAAAGCGTCATCCACACCAAAGG CAAATGTCCCCAAGTGAGTGGGCTCCCCGCTGTCACTG
ID2	NM_002166.4	505-605	CGGATATCAGCATCCTGTCCTTGCAGGCTTCTGAATTCCCTTCTGAGTTAATGTCAAATGACAG CAAAGCACTGTGTGGCTGAATAAGCGGTGTTCATGA
ID3	NM_002167.3	195-295	AGGAAGCCTGTTTGCAATTTAAGCGGGCTGTGAACGCCCAGGGCCGGCGGGGGCAGGGCCG AGGCGGGCCATTTTGAATAAAGAGGCGTGCCTTCCAGGC
IDO1	NM_002164.3	50-150	CTATTATAAGATGCTCTGAAAACTCTTCAGACACTGAGGGGCACCAGAGGAGCAGACTACAAG AATGGCACACGCTATGGAAAACTCCTGGACAATCAGT

IFNA1	NM_024013.1	585-685	ATCCCTCTCTTTATCAACAAACTTGCAAGAAAGATTAAGGAGGAAGGAATAACATCTGGTCCAA CATGAAAACAATTCTTATTGACTCATACACCAGGTC
IFNG	NM_000619.2	970-1070	ATACTATCCAGTTACTGCCGGTTTGAAAATATGCCTGCAATCTGAGCCAGTGCTTTAATGGCAT GTCAGACAGAACTTGAATGTGTCAGGTGACCCTGAT
IFNGR1	NM_000416.1	1140-1240	CCCGGGCAGCCATCTGACTCCAATAGAGAGAGAGAGTTCTTCACCTTTAAGTAGTAACCAGTC TGAACCTGGCAGCATCGCTTTAAACTCGTATCACTCC
IGF1R	NM_000875.2	455-555	TCGGGGGGCCATCAGGATTGAGAAAAATGCTGACCTCTGTTACCTCTCCACTGTGGACTGGTC CCTGATCCTGGATGCGGTGTCCAATAACTACATTGTG
IKZF1	NM_006060.3	4485-4585	CCGCTGTGTACTACTGTGTGCCTAGATTCCATGCACTCTCGTTGTGTTTGAAGTAAATATTGGA GACCGGAGGGTAACAGGTTGGCCTGTTGATTACAGC
IKZF2	NM_001079526.1	945-1045	CCATGTACCTCCTATGGAAGATTGTAAGGAACAAGAGCCTATTATGGACAACAATATTTCTCTG GTGCCTTTTGAGAGACCTGCTGTCATAGAGAAGCTC
IL10	NM_000572.2	230-330	AAGGATCAGCTGGACAACTTGTTGTTAAAGGAGTCCTTGCTGGAGGACTTTAAGGGTTACCTG GGTTGCCAAGCCTTGTCTGAGATGATCCAGTTTTACC
IL10RA	NM_001558.2	150-250	TGCCCAGCCCTCCGTCTGTGTGGTTTGAAGCAGAAATTTTCCACCACATCCTCCACTGGACAC CCATCCCAAATCAGTCTGAAAGTACCTGCTATGAAGT
IL12A	NM_000882.2	775-875	CTTTCTAGATCAAAACATGCTGGCAGTTATTGATGAGCTGATGCAGGCCCTGAATTTCAACAGT GAGACTGTGCCACAAAAATCCTCCCTTGAAGAACCG
IL12B	NM_002187.2	1435-1535	GCAAGGCTGCAAGTACATCAGTTTTATGACAATCAGGAAGAATGCAGTGTTCTGATACCAGTG CCATCATACACTTGTGATGGATGGGAACGCAAGAGAT
IL12RB1	NM_005535.1	1292-1392	AGGAAAAGTGTTACTACATTACCATCTTTGCCTCTGCGCACCCCGAGAAGCTCACCTTGTGGT CTACGGTCCTGTCCACCTACCACTTTGGGGGCAATGC
IL12RB2	NM_001559.2	1315-1415	CCTCCGTGGGACATTAGAATCAAATTTCAAAAGGCTTCTGTGAGCAGATGTACCCTTTATTGGA GAGATGAGGGACTGGTACTGCTTAATCGACTCAGAT
IL13	NM_002188.2	516-616	TTTCTTTCTGATGTCAAAAATGTCTTGGGTAGGCGGGAAGGAGGGTTAGGGAGGGGTAAAATT CCTTAGCTTAGACCTCAGCCTGTGCTGCCCGTCTTCA
IL15	NM_172174.1	1685-1785	AGGGTGATAGTCAAATTATGTATTGGTGGGGCTGGGTACCAATGCTGCAGGTCAACAGCTATG CTGGTAGGCTCCTGCCAGTGTGGAACCACTGACTACT
IL15RA	NM_002189.2	39-139	CGCTCGCCCGGGGAGTCCAGCGGTGTCCTGTGGAGCTGCCGCCATGGCCCCGCGGCGGGC GCGCGGCTGCCGGACCCTCGGTCTCCCGGCGCTGCTACTG
IL17A	NM_002190.2	240-340	TACTACAACCGATCCACCTCACCTTGGAATCTCCACCGCAATGAGGACCCTGAGAGATATCCC TCTGTGATCTGGGAGGCAAAGTGCCGCCACTTGGGCT
IL17F	NM_052872.3	210-310	GCCCGCCTGTGCCAGGAGGTAGTATGAAGCTTGACATTGGCATCATCAATGAAAACCAGCGC GTTTCCATGTCACGTAACATCGAGAGCCGCTCCACCTC
IL17RA	NM_014339.4	3020-3120	CTACTATGTGGCGGGCATTTGGGATACCAAGATAAATTGCATGCGGCATGGCCCCAGCCATGA AGGAACTTAACCGCTAGTGCCGAGGACACGTTAAACG
IL18	NM_001562.2	48-148	GACAGTCAGCAAGGAATTGTCTCCCAGTGCATTTTGCCCTCCTGGCTGCCAACTCTGGCTGCT AAAGCGGCTGCCACCTGCTGCAGTCTACACAGCTTCG
IL18R1	NM_003855.2	2025-2125	GAATGAGGGGATTTTAAGTGTCTGAAGAGGCATTTTCTAGGGACCAGTGGGTGACTGAGTAAC TGAAATGCTGCTTTCACTCCCTAACACCATGGATCTG
IL18RAP	NM_003853.2	2412-2512	GCTTGATGGACAATGGAGTGGGATTGAGACTGTGGTTTAGAGCCTTTGATTTCCTGGACTGGA CTGACGGCGAGTGAATTCTCTAGACCTTGGGTACTTT
IL1A	NM_000575.3	1085-1185	ACTCCATGAAGGCTGCATGGATCAATCTGTGTCTCTGAGTATCTCTGAAACCTCTAAAACATCC AAGCTTACCTTCAAGGAGAGCATGGTGGTAGTAGCA
IL1B	NM_000576.2	840-940	GGGACCAAAGGCGGCCAGGATATAACTGACTTCACCATGCAATTTGTGTCTTCCTAAAGAGAG CTGTACCCAGAGAGTCCTGTGCTGAATGTGGACTCAA
IL2	NM_000586.2	300-400	AGGATGCAACTCCTGTCTTGCATTGCACTAAGTCTTGCACTTGTCACAAACAGTGCACCTACTT CAAGTTCTACAAAGAAAACACAGCTACAACTGGAGC
IL21R	NM_021798.2	2080-2180	CGTGTTTGTGGTCAACAGATGACAACAGCCGTCCTCCCTCCTAGGGTCTTGTGTTGCAAGTTG GTCCACAGCATCTCCGGGGCTTTGTGGGATCAGGGCA
IL22	NM_020525.4	319-419	CTATCTGATGAAGCAGGTGCTGAACTTCACCCTTGAAGAAGTGCTGTTCCCTCAATCTGATAG GTTCCAGCCTTATATGCAGGAGGTGGTGCCCTTCCTG
IL23A	NM_016584.2	411-511	CAGGGACAACAGTCAGTTCTGCTTGCAAAGGATCCACCAGGGTCTGATTTTTTATGAGAAGCT GCTAGGATCGGATATTTTCACAGGGGAGCCTTCTCTG
IL23R	NM_144701.2	710-810	AACTGCAAATTCACCTGGATGATATAGTGATACCTTCTGCAGCCGTCATTTCCAGGGCTGAGA CTATAAATGCTACAGTGCCCAAGACCATAATTTATTG
IL27	NM_145659.3	143-243	CAGGAGCTGCGGAGGGAGTTCACAGTCAGCCTGCATCTCGCCAGGAAGCTGCTCTCCGAGGT TCGGGGCCAGGCCCACCGCTTTGCGGAATCTCACCTGC
IL2RA	NM_000417.1	1000-1100	CTTGGTAAGAAGCCGGGAACAGACAACAGAAGTCATGAAGCCCAAGTGAAATCAAAGGTGCT AAATGGTCGCCCAGGAGACATCCGTTGTGCTTGCCTGC
IL2RB	NM_000878.2	1980-2080	GTCCTGCTGCCCGAGCCAGGAACTGTGTGTGTTGCAGGGGGGCAGTAACTCCCCAACTCCCT CGTTAATCACAGGATCCCACGAATTTAGGCTCAGAAGC
IL2RG	NM_000206.1	595-695	CCACAGCTGGACTGAACAATCAGTGGATTATAGACATAAGTTCTCCTTGCCTAGTGTGGATGG GCAGAAACGCTACACGTTTCGTGTTCGGAGCCGCTTT
IL4	NM_000589.2	625-725	GACACTCGCTGCCTGGGTGCGACTGCACAGCAGTTCCACAGGCACAAGCAGCTGATCCGATT CCTGAAACGGCTCGACAGGAACCTCTGGGGCCTGGCGG
IL4R	NM_000418.2	705-805	ATCATCTCACCTATGCAGTCAACATTTGGAGTGAAAACGACCCGGCAGATTTCAGAATCTATAA CGTGACCTACCTAGAACCCTCCCTCCGCATCGCAGC
IL5	NM_000879.2	105-205	CCACAGAAATTCCCACAAGTGCATTGGTGAAAGAGACCTTGGCACTGCTTTCTACTCATCGAA CTCTGCTGATAGCCAATGAGACTCTGAGGATTCCTGT

IL6	NM_000600.1	220-320	TGACAAACAAATTCGGTACATCCTCGACGGCATCTCAGCCCTGAGAAAGGAGACATGTAACAA GAGTAACATGTGTGAAAGCAGCAAAGAGGCACTGGCA
IL6R	NM_000565.2	993-1093	CTTTCTACATAGTGTCCATGTGCGTCGCCAGTAGTGTCGGGAGCAAGTTCAGCAAAACTCAAA CCTTTCAGGGTTGTGGAATCTTGCAGCCTGATCCGCC
IL7R	NM_002185.2	1610-1710	TTGCTTTGACCACTCTTCCTGAGTTCAGTGGCACTCAACATGAGTCAAGAGCATCCTGCTTCTA CCATGTGGATTTGGTCACAAGGTTTAAGGTGACCCA
IL9	NM_000590.1	300-400	AAGTACTAAAGAACAACAAGTGTCCATATTTTTCCTGTGAACAGCCATGCAACCAAACCACGGC AGGCAACGCGCTGACATTTCTGAAGAGTCTTCTGGA
IRF1	NM_002198.1	510-610	CTGTGCGAGTGTACCGGATGCTTCCACCTCTCACCAAGAACCAGAGAAAAGAAAGAAAGTCGA AGTCCAGCCGAGATGCTAAGAGCAAGGCCAAGAGGAA
IRF2	NM_002199.2	1375-1475	CAGTACCTGGAGCTTCTCTTTAACTCAGGACTCCAGCCCATTGGTAGACGTGTGTTTCTAGAG CCTGCTGGATCTCCCAGGGCTACTCACTCAAGTTCAA
IRF4	NM_002460.1	325-425	GGGCACTGTTTAAAGGAAAGTTCCGAGAAGGCATCGACAAGCCGGACCCTCCCACCTGGAAG ACGCGCCTGCGGTGCGCTTTGAACAAGAGCAATGACTT
ITCH	NM_031483.4	155-255	ACTGTGAGAACTTCAGGTTTTCCAACCTATTGGTGGTATGTCTGACAGTGGATCACAACTTGGT TCAATGGGTAGCCTCACCATGAAATCACAGCTTCAG
ITGA1	NM_181501.1	1875-1975	AAGTGGCAAGACTATAAGGAAAGAGTATGCACAACGTATTCCATCAGGTGGGGATGGTAAGAC ACTGAAATTTTTTGGCCAGTCTATCCACGGAGAAATG
ITGA4	NM_000885.4	975-1075	GCCCACTGCCAACTGGCTCGCCAACGCTTCAGTGATCAATCCCGGGGCGATTTACAGATGCA GGATCGGAAAGAATCCCGGCCAGACGTGCGAACAGCTC
ITGA5	NM_002205.2	925-1025	AGAAGACTTTGTTGCTGGTGTGCCCAAAGGGAACCTCACTTACGGCTATGTCACCATCCTTAA TGGCTCAGACATTCGATCCCTCTACAACTTCTCAGGG
ITGAL	NM_002209.2	3905-4005	GTGAGGGCTTGTCATTACCAGACGGTTCACCAGCCTCTCTTGGTTTCCTTCCTTGGAAGAGAA TGTCTGATCTAAATGTGGAGAAACTGTAGTCTCAGGA
ITGAM	NM_000632.3	515-615	GCCCTCCGAGGGTGTCCTCAAGAGGATAGTGACATTGCCTTCTTGATTGATGGCTCTGGTAGC ATCATCCCACATGACTTTCGGCGGATGAAGGAGTTTG
ITGAX	NM_000887.3	700-800	CCCCTCAGCCTGTTGGCTTCTGTTCACCAGCTGCAAGGGTTTACATACACGGCCACCGCCATC CAAAATGTCGTGCACCGATTGTTCCATGCCTCATATG
ITGB1	NM_033666.2	2000-2100	TTTTAACATTACCAAGGTAGAAAGTCGGGACAAATTACCCCAGCCGGTCCAACCTGATCCTGT GTCCCATTGTAAGGAGAAGGATGTTGACGACTGTTGG
ITGB7	NM_000889.1	1278-1378	CAACGTGGTACAGCTCATCATGGATGCTTATAATAGCCTGTCTTCCACCGTGACCCTTGAACA CTCTTCACTCCCTCCTGGGGTCCACATTTCTTACGAA
ITK	NM_005546.3	3430-3530	GCCAGTAAAGAAGTCAGTATAGAACCACTAGCGAATAGTGTTGCTCTGGCACAGACCACTGTG GTTGATGGCATGGCCCTCCAACTTGGAATAGGATTTT
JAK1	NM_002227.1	285-385	GAGAACACCAAGCTCTGGTATGCTCCAAATCGCACCATCACCGTTGATGACAAGATGTCCCTC CGGCTCCACTACCGGATGAGGTTCTATTTCACCAATT
JAK2	NM_004972.2	455-555	CTCCTCCCGCGACGGCAAATGTTCTGAAAAAGACTCTGCATGGGAATGGCCTGCCTTACGATG ACAGAAATGGAGGGAACATCCACCTCTTCTATATATC
JAK3	NM_000215.2	1715-1815	GTGCTGCTGAAGGTCATGGATGCCAAGCACAAGAACTGCATGGAGTCATTCCTGGAAGCAGC GAGCTTGATGAGCCAAGTGTCGTACCGGCATCTCGTGC
JUN	NM_002228.3	140-240	ACACAGCCAGCCAGCCAGGTCGGCAGTATAGTCCGAACTGCAAATCTTATTTTCTTTTCACCTT CTCTCTAACTGCCCAGAGCTAGCGCCTGTGGCTCCC
JUNB	NM_002229.2	1155-1255	GCGCGCCTGGAGGACAAGGTGAAGACGCTCAAGGCCGAGAACGCGGGGCTGTCGAGTACCG CCGGCCTCCTCCGGGAGCAGGTGGCCCAGCTCAAACAGA
KIR2DL1	NM_014218.2	881-981	GCAGGAAACAGAACAGCGAATAGCGAGGACTCTGATGAACAAGACCCTCAGGAGGTGACATA CACACAGTTGAATCACTGCGTTTTCACACAGAGAAAAA
KIR2DL2	NM_014219.2	814-914	TCTCCTTCATCGCTGGTGCTCCAACAAAAAAAATGCTGCGGTAATGGACCAAGAGTCTGCAGG GAACAGAACAGCGAATAGCGAGGACTCTGATGAACAA
KIR2DL3	NM_015868.2	741-841	CTCCGAAACCGGTAACCCCAGACACCTGCATGTTCTGATTGGGACCTCAGTGGTCATCATCCT CTTCATCCTCCTCCTCTTCTTTCTCCTTCATCGCTGG
KIR2DL4	NM_002255.5	15-115	GCGTCCTGGCAGCAGAAGCTGCACCATGTCCATGTCACCCACGGTCATCATCCTGGCATGTC TTGGGTTCTTCTTGGACCAGAGTGTGTGGGCACACGTG
KIR2DL5A	NM_020535.3	1451-1551	GACACGTGCTGTTCCACCTTCCCTCATGCTGTTTCACCTTTCCTCAGACTATTTTCCAGCCTTC TGTCAGTCAGCAGTGAAACTTATAAAATTTTTTGTG
KIR2DS1	NM_014512.1	698-798	CTTCACCCACTGAACCAAGCTCCGAAACCGGTAACCCCAGACACCTACATGTTCTGATTGGGA CCTCAGTGGTCAAAATCCCTTTCACCATCCTCCTCTT
KIR2DS2	NM_012312.2	856-956	CAAGAGCCTGCAGGGAACAGAACAGTGAACAGCGAGGATTCTGATGAACAAGACCATCAGGA GGTGTCATACGCATAATTGGATCACTGTGTTTTCACAC
KIR2DS3	NM_012313.1	693-793	GGCCTTCACCCACTGAACCAAGCTCCAAAACCGGTAACCCCAGACACCTACACGTTCTGATTG GGACCTCAGTGGTCAAACTCCCTTTCACCATCCTCCT
KIR2DS4	NM_012314.3	1427-1527	ACATACAAGAGGCTGCCTCTTAACACAGCACTTAGACACGTGCTGTTCCACCTCCCTTCAGAC TATCTTTCAGCCTTCTGCCAGCAGTAAAACTTATAAA
KIR2DS5	NM_014513.2	204-304	CTTCCTTCTGCACAGAGAGGGGACGTTTAACCACACTTTGCGCCTCATTGGAGAGCACATTGA TGGGGTCTCCAAGGGCAACTTCTCCATCGGTCGCATG
KIR3DL1	NM_013289.2	1054-1154	CCAAATCTGGTAACCCCAGACACCTGCACATTCTGATTGGGACCTCAGTGGTCATCATCCTCT TCATCCTCCTCCTCTTCTTTCTCCTTCATCTCTGGTG
KIR3DL2	NM_006737.2	884-984	TGCCACCCACGGAGGGACCTACAGATGCTTCGGCTCTTTCCGTGCCCTGCCCTGCGTGTGGT CAAACTCAAGTGACCCACTGCTTGTTTCTGTCACAGGA
KIR3DL3	NM_153443.3	508-608	CCTTGCGCCTCGTTGGACAGCTCCACGATGCGGGTTCCCAGGTCAACTATTCCATGGGTCCC ATGACACCTGCCCTTGCAGGGACCTACAGATGCTTTGG

KIR3DS1	NM_001083539.1	1000-1100	CTCCAAATCTGGTAACCTCAGACACCTGCACATTCTGATTGGGACCTCAGTGGTCAAAATCCCTTTCACCATCCTCCTCTTCTTTCTCCTTCATCGCTGG
KIT	NM_000222.1	5-105	CATCGCAGCTACCGCGATGAGAGGCGCTCGCGGCGCCTGGGATTTTCTCTGCGTTCTGCTCCTACTGCTTCGCGTCCAGACAGGCTCTTCTCAACCATCT
KLF10	NM_005655.1	570-670	GCTCAGGCAACAAGTGTGATTCGTCATACAGCTGATGCCCAGCTATGTAACCACCAGACCTGCCCAATGAAAGCAGCCAGCATCCTCAACTATCAGAACA
KLF2	NM_016270.2	1015-1115	GGAAGTTTGCGCGCTCAGACGAGCTCACGCGCCACTACCGAAAGCACACGGGCCACCGGCCATTCCAGTGCCATCTGTGCGATCGTGCCTTCTCGCGCTC
KLF4	NM_004235.4	1980-2080	CGAGCATTTTCCAGGTCGGACCACCTCGCCTTACACATGAAGAGGCATTTTTAAATCCCAGACAGTGGATATGACCCACACTGCCAGAAGAGAATTCAGT
KLF6	NM_001008490.1	1165-1265	GGGATGCGTGTTCCAGCCAAAGCATGCCGTTCTGCACCCTACCCAGTTGCCTCCAGGGCCTCTCCTTGGAAGGTCTTTTGAGGGCTAAAAAGGTCCTGTA
KLF7	NM_001270943.1	1546-1646	GTACTATTGAGATCTTTCGCGTCGATCCCAACGGCCTTAGCGGCGGCAGACTGGAATAACACCTTACACCTTTCTGGCCTGCATTTCTGTAGACTTCACT
KLRAP1	NR_028045.1	414-514	CCTTCAGAGTCACAGAATAGATTAAGGCCTGATGATACTCAAAGGCCTGGGAAAACTGATGACAAAGAATTTTCAGTGCCCTGGCACCTCATTGCAGTGA
KLRB1	NM_002258.2	85-185	TGAGTTAAACTTACCCACAGACTCAGGCCCAGAAAGTTCTTCACCTTCATCTCTTCCTCGGGATGTCTGTCAGGGTTCACCTTGGCATCAATTTGCCCTG
KLRC1	NM_002259.3	335-435	ACCTATCACTGCAAAGATTTACCATCAGCTCCAGAGAAGCTCATTGTTGGGATCCTGGGAATTATCTGTCTTATCTTAATGGCCTCTGTGGTAACGATAG
KLRC2	NM_002260.3	942-1042	TATGTGAGTCAGCTTATAGGAAGTACCAAGAACAGTCAAACCCATGGAGACAGAAAGTAGAATAGTGGTTGCCAATGTCTCAGGGAGGTTGAAATAGGAG
KLRC3	NM_002261.2	760-860	ACTCCTGAGCTCAAGAAATCAACACATCTTGGCCTCCCAAGTTGCTGGGATTACTGACACAAGCCACCGCCCCTGAGTGCTCATGTACCATTTAGCTTGT
KLRC4	NM_013431.2	29-129	TTATATTGGTCAACAGCAAAATGAACATTACTACTCAGCCTCCAACACATGCAGTTTGCCTATACCAGGGATCCTGTCAAAATATACACCACTTATAGCT
KLRD1	NM_002262.3	542-642	AGCCTGCTTCAGCTTCAAAACACAGATGAACTGGATTTTATGAGCTCCAGTCAACAATTTTACTGGATTGGACTCTCTTACAGTGAGGAGCACACCGCCT
KLRF1	NM_016523.1	275-375	AAAAAGGAAGTTGTTCAAATGCCACTCAGTATGAGGACACTGGAGATCTAAAAGTGAATAATGGCACAAGAAGAAATATAAGTAATAAGGACCTTTGTGC
KLRG1	NM_005810.3	45-145	TGCCTACGGCAACCCAAGCCCAGAATGACTATGGACCACAGCAAAAATCTTCCTCTTCCAGGCCTTCTTGTTCTTGCCTTGTGGCAATAGCTTTGGGGCT
KLRK1	NM_007360.1	760-860	GGACCAGGATTTACTTAAACTGGTGAAGTCATATCATTGGATGGGACTAGTACACATTCCAACAAATGGATCTTGGCAGTGGGAAGATGGCTCCATTCTC
LAG3	NM_002286.5	1735-1835	CTTTTGGTGACTGGAGCCTTTGGCTTTCACCTTTGGAGAAGACAGTGGCGACCAAGACGATTTTCTGCCTTAGAGCAAGGGATTCACCCTCCGCAGGCTC
LAIR1	NM_002287.3	1195-1295	GCACCTGAGGGTAGAAAGTCACTCTAGGAAAAGCCTGAAGCAGCCATTTGGAAGGCTTCCTGTTGGATTCCTCTTCATCTAGAAAGCCAGCCAGGCAGCT
LAT	NM_001014987.1	1290-1390	TGTGTAATAGAATAAAGGCCTGCGTGTGTCTGTGTTGAGCGTGCGTCTGTGTGTGCCTGTGTGCGAGTCTGAGTCAGAGATTTGGAGATGTCTCTGTGTG
LAT2	NM_014146.3	1863-1963	TGCAGAGCTGATTAAACAGTGTTGTGACTGTCTCATGGGAAGAGCTGGGGCCCAGAGGGACCTTGAGTCAGAAATGTTGCCAGAAAAAGTATCTCCTCCA
LCK	NM_005356.2	1260-1360	ATTAAGTGGACAGCGCCAGAAGCCATTAACTACGGGACATTCACCATCAAGTCAGATGTGTGGTCTTTTGGGATCCTGCTGACGGAAATTGTCACCCACG
LDHA	NM_005566.1	985-1085	CAGAATGGAATCTCAGACCTTGTGAAGGTGACTCTGACTTCTGAGGAAGAGGCCCGTTTGAAGAAGAGTGCAGATACACTTTGGGGGATCCAAAAGGAGC
LEF1	NM_016269.3	1165-1265	CCGTCACACATCCCATCAGATGTCAACTCCAAACAAGGCATGTCCAGACATCCTCCAGCTCCTGATATCCCTACTTTTTATCCCTTGTCTCCGGGTGGTG
LGALS1	NM_002305.3	60-160	GGTGCGCCTGCCCGGGAACATCCTCCTGGACTCAATCATGGCTTGTGGTCTGGTCGCCAGCAACCTGAATCTCAAACCTGGAGAGTGCCTTCGAGTGCGA
LGALS3	NM_002306.2	120-220	CAGCCGTCCGGAGCCAGCCAACGAGCGGAAAATGGCAGACAATTTTTCGCTCCATGATGCGTTATCTGGGTCTGGAAACCCAAACCCTCAAGGATGGCCT
LIFR	NM_002310.3	2995-3095	CCTATTGTCCACCCATCATTGAGGAAGAAATACCAAACCCAGCCGCAGATGAAGCTGGAGGGACTGCACAGGTTATTTACATTGATGTTCAGTCGATGTA
LILRB1	NM_001081637.1	2332-2432	AGCTGAGAAAACTAAGTCAGAAAGTGCATTAAACTGAATCACAATGTAAATATTACACATCAAGCGATGAAACTGGAAAACTACAAGCCACGAATGAATG
LOC282997	NR_026932.1	665-765	TGATCACATTCTACCTGGCATTATTTCATCTGAGTCCCTGTCCTAGCCCTTCTGCCCATTAGACTGTAACCTTGTTTAGGGAAAGACCTGTGTCTTACTC
LRP5	NM_002335.1	2515-2615	TGGACACCAACATGATCGAGTCGTCCAACATGCTGGGTCAGGAGCGGGTCGTGATTGCCGACGATCTCCCGCACCCGTTCGGTCTGACGCAGTACAGCGA
LRP6	NM_002336.1	2185-2285	CTTAGATTATCCAGAAGGCATGGCAGTAGACTGGCTTGGGAAGAACTTGTACTGGGCAGACACAGGAACGAATCGAATTGAGGTGTCAAAGTTGGATGGG
LRRC32	NM_005512.2	3470-3570	CACCCTGGTGTGGGTTCTCCTGTTCTCTCTGTGCTCTTGCATTCTCTCATTCCCTTTTCCTCTATTGAGCAGAGCCTGGAGTTTGAGACTATGGAATCCA
LTA	NM_000595.2	885-985	CTGATCAAGTCACCGGAGCTTTCAAAGAAGGAATTCTAGGCATCCCAGGGGACCACACCTCCCTGAACCATCCCTGATGTCTGTCTGGCTGAGGATTTCA
LTBR	NM_002342.1	1435-1535	CTAACAGGGGCCCAAGGAACCAATTTATCACCCATGACTGACGGAGTCTGAGAAAAGGCAGAAGAAGGGGGGCACAAGGGCACTTTCTCCCTTGAGGCTG
LYN	NM_002350.1	1285-1385	TCCTGAAGAGCGATGAAGGTGGCAAAGTGCTGCTTCCAAAGCTCATTGACTTTTCTGCTCAGATTGCAGAGGGAATGGCATACATCGAGCGGAAGAACTA

MAD1L1	NM_003550.2	306-406	GAAGACCTGGGGGAAAACACCATGGTTTTATCCACCCTGAGATCTTTGAACAACTTCATCTCTC AGCGTGTGGAGGGAGGCTCTGGACTGGATATTTCTA
MAP2K1	NM_002755.2	970-1070	ACGGAATGGACAGCCGACCTCCCATGGCAATTTTTGAGTTGTTGGATTACATAGTCAACGAGC CTCCTCCAAAACTGCCCAGTGGAGTGTTCAGTCTGGA
MAPK14	NM_001315.1	450-550	TGGGCTCTGGCGCCTATGGCTCTGTGTGTGCTGCTTTTGACACAAAAACGGGGTTACGTGTG GCAGTGAAGAAGCTCTCCAGACCATTTCAGTCCATCAT
MAPK3	NM_002746.2	580-680	AACGTGCTCCACCGAGATCTAAAGCCCTCCAACCTGCTCATCAACACCACCTGCGACCTTAAG ATTTGTGATTTCGGCCTGGCCCGGATTGCCGATCCTG
MAPK8	NM_139049.1	945-1045	TCTCTGTAGATGAAGCTCTCCAACACCCGTACATCAATGTCTGGTATGATCCTTCTGAAGCAGA AGCTCCACCACCAAAGATCCCTGACAAGCAGTTAGA
MBD2	NM_003927.3	2015-2115	ATTTACATTCAACTCTGATCCCTGGGCCTTAGGTTTGACATGGAGGTGGAGGAAGATAGCGCA TATATTTGCAGTATGAACTATTGCCTCTGGACGTTGT
MCL1	NM_021960.3	1260-1360	GCTGTAACTTCCTAGAGTTGCACCCTAGCAACCTAGCCAGAAAAGCAAGTGGCAAGAGGATTA TGGCTAACAAGAATAAATACATGGGAAGAGTGCTCCC
MIF	NM_002415.1	319-419	TCCTACAGCAAGCTGCTGTGCGGCCTGCTGGCCGAGCGCCTGCGCATCAGCCCGGACAGGG TCTACATCAACTATTACGACATGAACGCGGCCAATGTGG
MMP14	NM_004995.2	1470-1570	GACAAGATTGATGCTGCTCTCTTCTGGATGCCCAATGGAAAGACCTACTTCTTCCGTGGAAAC AAGTACTACCGTTTCAACGAAGAGCTCAGGGCAGTGG
MPL	NM_005373.2	895-995	CAGTGGCACTTGGACTGCAATGCTTTACCTTGGACCTGAAGAATGTTACCTGTCAATGGCAGC AACAGGACCATGCTAGCTCCCAAGGCTTCTTCTACCA
MTOR	NM_004958.2	5095-5195	TTAGTGTTGCTCCTGGGAGTTGATCCGTCTCGGCAACTTGACCATCCTCTGCCAACAGTTCAC CCTCAGGTGACCTATGCCTACATGAAAAACATGTGGA
MXD1	NM_002357.2	880-980	GAGAATAAAGCTGCAGGACAGTCACAAGGCGTGTCTTGGTCTCTAAGAGAGTGGGCACTGCG GCTGTCTCCTTGAAGGTTCTCCCTGTTGGTTCTGATTA
MYB	NM_005375.2	3145-3245	AACTGTTGCATGGATCCTGTGTTTGCAACTGGGGAGACAGAAACTGTGGTTGATAGCCAGTCA CTGCCTTAAGAACATTTGATGCAAGATGGCCAGCACT
MYC	NM_002467.3	1610-1710	TCGGACACCGAGGAGAATGTCAAGAGGCGAACACACAACGTCTTGGAGCGCCAGAGGAGGA ACGAGCTAAAACGGAGCTTTTTTGCCCTGCGTGACCAGA
MYO6	NM_004999.3	6655-6755	AAGTTGGGGAGATGGCACCTTCTCAGAGGATTGTGAAAATATGAGGAAGAAACAAAACAGTGC ATGTAGGAGCACAGGGCCACACAAAGGCATTCTATTG
NANOG	NM_024865.2	1100-1200	CTACTCCATGAACATGCAACCTGAAGACGTGTGAAGATGAGTGAAACTGATATTACTCAATTTC AGTCTGGACACTGGCTGAATCCTTCCTCTCCCCTCC
NBEA	NM_015678.3	8645-8745	CTGAGAGCCCTTGAAGGACCAGAAAACTGCTTATTCCCACGCTTGATATCTGTCTCCAGCGAA GGCCACTGTATCATATACTATGAACGAGGGCGATTCA
NCAM1	NM_000615.5	1620-1720	GGTATTTGCCTATCCCAGTGCCACGATCTCATGGTTTCGGGATGGCCAGCTGCTGCCAAGCTC CAATTACAGCAATATCAAGATCTACAACACCCCCTCT
NCL	NM_005381.2	1492-1592	GAACAGAGATCGATGGGCGATCTATTTCCCTGTACTATACTGGAGAGAAAGGTCAAAATCAAG ACTATAGAGGTGGAAAGAATAGCACTTGGAGTGGTGA
NCR1	NM_001145457.1	145-245	TTTCATGGTTCCAAAGGAAAAGCAAGTGACCATCTGTTGCCAGGGAAATTATGGGGCTGTTGA ATACCAGCTGCACTTTGAAGGAAGCCTTTTTGCCGTG
NCR2	NM_004828.3	798-898	CTTCAACAGGTCACGGACCTTCCCTGGACCTCAGTTTCCTCACCTGTAGAGAGAGAAATATTAT ATCACACTGTTGCAAGGACTAAGATAAGCGATGATG
NCR3	NM_147130.1	50-150	GCATCTGTCCTCTCTCCTCAGGGAGGCAAGCATTTGATGCTCGAGGTCCCTGGCAGTTGTGGT CCTTGGCAAGTGATGTGTGAGTCCCGTGTGTCATAGG
NCRNA00185	NR_001544.2	143-243	GAGGCTGTCTGCCAACATCTTTCATCACTCTGCCTGCAACTATGAAAAATTTAGTTCTAAAAAAT GCAACCTTGCTAAATTGAGTACTAATAGGATTGGT
NEIL1	NM_024608.2	1675-1775	TTAGCAGGAGGCTCTCCTTGCTTGCACTCACCCTTTCTTATTGTCTTGCCCTGCATCTGGGGG TCTGAATTTTTGGGAGCAGGCAATATCTGAAGGTGCA
NEIL2	NM_145043.2	2570-2670	GCCCGGTGGTGTGTAGAGAAAAGCTGCTTGTTTACTCCTTAAGTCAATGTATTGGTGACTGTT GATTTGTTGAACAATTCAGGAATCAAGGGCTGTGGAG
NFAT5	NM_173214.1	3290-3390	CCCTGACAACTATTCAAACCCAGGACATCTCACAGCCTGGTACTTTTCCAGCAGTTTCTGCTTC TAGTCAGCTGCCCAACAGCGATGCACTATTGCAGCA
NFATC1	NM_172390.1	2510-2610	CCAGTACCAGCGTTTCACCTACCTTCCCGCCAACGGTAACGCCATCTTTCTAACCGTAAGCCG TGAACATGAGCGCGTGGGGTGCTTTTTCTAAAGACGC
NFATC2	NM_012340.3	1815-1915	GACGGACATTGGAAGAAAGAACACGCGGGTGAGACTGGTTTTCCGAGTTCACATCCCAGAGT CCAGTGGCAGAATCGTCTCTTTACAGACTGCATCTAAC
NFATC3	NM_004555.2	2190-2290	GTCCTTGAAGTTCCTCCATATCATAACCCAGCAGTTACAGCTGCAGTGCAGGTGCACTTTTATC TTTGCAATGGCAAGAGGAAAAAAAGCCAGTCTCAAC
NFKB1	NM_001165412.1	2305-2405	CTTGGGTAACTCTGTTTTGCACCTAGCTGCCAAAGAAGGACATGATAAAGTTCTCAGTATCTTA CTCAAGCACAAAAAGGCAGCACTACTTCTTGACCAC
NOS2	NM_000625.4	605-705	TTGCCTGGGGTCCATTATGACTCCCAAAAGTTTGACCAGAGGACCCAGGGACAAGCCTACCC CTCCAGATGAGCTTCTACCTCAAGCTATCGAATTTGTC
NOTCH1	NM_017617.3	735-835	CTGCCAGGCTTCACCGGCCAGAACTGTGAGGAAAATATCGACGATTGTCCAGGAAACAACTG CAAGAACGGGGGTGCCTGTGTGGACGGCGTGAACACCT
NR3C1	NM_001018077.1	1665-1765	GCTTTCTCCTCTGGCGGGAGAAGACGATTCATTCCTTTTGGAAGGAAACTCGAATGAGGACTG CAAGCCTCTCATTTTACCGGACACTAAACCCAAAATT
NR4A1	NM_002135.3	155-255	CGGCCGGGTAGGGTGCAGCCTGAGGCTTGTTCAGCAGAACAGGTGCAAGCCACATTGTTGCC AAGACCTGCCTGAAGCCGGATTCTCCCCACTGCCTCCT
NREP	NM_001142474.1	990-1090	AAACTCATTGTTTCCTTGTGGTAAGTGACCGAGATGCTGCCACAGGACCTGAGACACTGATGA ATGGTGCTATTTTGGACTTTCAACATGCTCCTTGGCG

NRIP1	NM_003489.2	335-435	TGACTCATGGAGAAGAGCTTGGCTCTGATGTGCACCAGGATTCTATTGTTTTAACTTACCTAGA AGGATTACTAATGCATCAGGCAGCAGGGGGATCAGG
NRP1	NM_003873.5	370-470	GCCTCGCTGCTTTCTTTTCTCCAAGACGGGCTGAGGATTGTACAGCTCTAGGCGGAGTTGGG GCTCTTCGGATCGCTTAGATTCTCCTCTTTGCTGCATT
NT5E	NM_002526.2	1214-1314	ATTCGGGTTTTGAAATGGATAAACTCATCGCTCAGAAAGTGAGGGGTGTGGACGTCGTGGTG GGAGGACACTCCAACACATTTCTTTACACAGGCAATCC
OAZ1	NM_004152.2	313-413	GGTGGGCGAGGGAATAGTCAGAGGGATCACAATCTTTCAGCTAACTTATTCTACTCCGATGAT CGGCTGAATGTAACAGAGGAACTAACGTCCAACGACA
OPTN	NM_001008211.1	625-725	TGAAGCTAAATAATCAAGCCATGAAAGGGAGATTTGAGGAGCTTTCGGCCTGGACAGAGAAAC AGAAGGAAGAACGCCAGTTTTTTGAGATACAGAGCAA
P2RX7	NM_002562.4	340-440	AGTTGGTGCACAGTGTCTTTGACACCGCAGACTACACCTTCCCTTTGCAGGGGAACTCTTTCT TCGTGATGACAAACTTTCTCAAAACAGAAGGCCAAGA
PAX5	NM_016734.1	2288-2388	CTCCAAGAGGAGCACACTTTGGGGAGATGTCCTGGTTTCCTGCCTCCATTTCTCTGGGACCGA TGCAGTATCAGCAGCTCTTTTCCAGATCAAAGAACTC
PDCD1	NM_005018.1	175-275	CTTCTTCCCAGCCCTGCTCGTGGTGACCGAAGGGGACAACGCCACCTTCACCTGCAGCTTCT CCAACACATCGGAGAGCTTCGTGCTAAACTGGTACCGC
PDCD1LG2	NM_025239.3	235-335	TGTGGAGCTGTGGCAAGTCCTCATATCAAATACAGAACATGATCTTCCTCCTGCTAATGTTGAG CCTGGAATTGCAGCTTCACCAGATAGCAGCTTTATT
PDE3A	NM_000921.3	3010-3110	CTGGCCAACCTTCAGGAATCCTTCATCTCTCACATTGTGGGGCCTCTGTGCAACTCCTATGATT CAGCAGGACTAATGCCTGGAAAATGGGTGGAAGACA
PDE4A	NM_001111307.1	3855-3955	AATAATGGTGTATACCCTCATTCTCATTCCTGGGCAGCCCTTCCTTCCACCCTGGCACCAAAAT AATTTCTCCTCCATCCGTACCTTGCCTAGCCTCTCC
PDE7A	NM_002604.2	2210-2310	GTAGCTCAACAAGGAATAGAGGGAGGAGTGTAATTTTGGTAGCTGGTGTTGAATAGGGCCTTT GAGAATCAGACTGAACACAGTGAAATATGTGCCCAAA
PDK1	NM_002610.3	1170-1270	TGGATTGCCCATATCACGTCTTTACGCACAATACTTCCAAGGAGACCTGAAGCTGTATTCCCTA GAGGGTTACGGGACAGATGCAGTTATCTACATTAAG
PDXK	NM_003681.3	580-680	TCCCGGAGGACCTCCTTCCCGTCTACAAAGAAAAAGTGGTGCCGCTTGCAGACATTATCACGC CCAACCAGTTTGAGGCCGAGTTACTGAGTGGCCGGAA
PECAM1	NM_000442.3	1365-1465	ATCTGCACTGCAGGTATTGACAAAGTGGTCAAGAAAAGCAACACAGTCCAGATAGTCGTATGT GAAATGCTCTCCCAGCCCAGGATTTCTTATGATGCCC
PHACTR2	NM_001100164.1	8350-8450	GGCAGAATGCCACTCTACCCTCAGGTCAATTTTATGGTATATGAAAATGCCAGTAATATTTGTG CCACTTGCCAACTCGGGGGAGGAGGGGCTTTTCCCT
PHC1	NM_004426.2	2905-3005	ATACAGCTCCACCTACACCGGAATTACATGGCATCAACCCTGTGTTCCTGTCCAGTAATCCCA GCCGTTGGAGTGTAGAGGAGGTGTACGAGTTTATTGC
POLR1B	NM_019014.3	3320-3420	GGAGAACTCGGCCTTAGAATACTTTGGTGAGATGTTAAAGGCTGCTGGCTACAATTTCTATGG CACCGAGAGGTTATATAGTGGCATCAGTGGGCTAGAA
POLR2A	NM_000937.2	3775-3875	TTCCAAGAAGCCAAAGACTCCTTCGCTTACTGTCTTCCTGTTGGGCCAGTCCGCTCGAGATGC TGAGAGAGCCAAGGATATTCTGTGCCGTCTGGAGCAT
POP5	NM_015918.3	560-660	GCTTCAGGCCCACTTGTTGAACAGAACAATCTGGGTAGCAACAGCATCTTCCACAGTTTTCCA AACTGGATAGCTGCCAACCAGCAGACATTACCCACTT
POU5F1	NM_002701.4	1225-1325	AAGTTCTTCATTCACTAAGGAAGGAATTGGGAACACAAAGGGTGGGGGCAGGGGAGTTTGGG GCAACTGGTTGGAGGGAAGGTGAAGTTCAATGATGCTC
PPARA	NM_001001928.2	5220-5320	GGGTGTGTTTGCTATACGAACATAATGGACGTGAAGTGGGGCAGAAACCCAGAACTCAGCATT CAAGGATGCCCAGGAGAGCTGTCCCTGTTTTAAAGAG
PPP2R1A	NM_014225.3	1440-1540	AACTTAACTCCTTGTGCATGGCCTGGCTTGTGGATCATGTATATGCCATCCGCGAGGCAGCCA CCAGCAACCTGAAGAAGCTAGTGGAAAAGTTTGGGAA
PRDM1	NM_182907.1	310-410	CATCCCTGCCAACCAGGAACTTCTTGTGTGGTATTGTCGGGACTTTGCAGAAAGGCTTCACTA CCCTTATCCCGGAGAGCTGACAATGATGAATCTCACA
PRF1	NM_005041.3	2120-2220	ACTGTTTTTCAGGGAGGTGGCTGGGTTTACACGCTAATCCCGATTCACCCTGTCCAAACTGCC TAAGCCCTCCGCCATTCTCAAGCCCTGCAGTCACAGC
PRKAA2	NM_006252.2	975-1075	ATAGTGGTGACCCTCAAGACCAGCTTGCAGTGGCTTATCATCTTATCATTGACAATCGGAGAAT AATGAACCAAGCCAGTGAGTTCTACCTCGCCTCTAG
PRKCQ	NM_006257.2	1325-1425	GATGGACGATGATGTTGAGTGCACGATGGTAGAGAAGAGAGTTCTTTCCTTGGCCTGGGAGC ATCCGTTTCTGACGCACATGTTTTGTACATTCCAGACC
PROM1	NM_006017.1	925-1025	AGCCTGCGGTCATCTCTCAATGACCCTCTGTGCTTGGTGCATCCATCAAGTGAAACCTGCAAC AGCATCAGATTGTCTCTAAGCCAGCTGAATAGCAACC
PTGER2	NM_000956.2	1410-1510	GTCAGAAGGAGCTACAAAACCTACCCTCAGTGAGCATGGTACTTGGCCTTTGGAGGAACAATC GGCTGCATTGAAGATCCAGCTGCCTATTGATTTAAGC
PTK2	NM_005607.3	1005-1105	GGTTCAAGCTGGATTATTTCAGTGGAACTGGCAATCGGCCCAGAAGAAGGAATCAGTTACCTA ACGGACAAGGGCTGCAATCCCACACATCTTGCTGACT
PTPN11	NM_002834.3	4650-4750	TAGTCCCTAGGTTGCTACGGCTTATCATGTGCTTGGTAAAAGGTGATCGCAGGTTCTCAGACG AGTTTACTTTACATGAGATGGAATCAGGCAGAGAGGC
PTPN4	NM_002830.2	705-805	TCGAGGCTTTTTTTCTCCAGCCGAGAGGACGCGGCTGTGATATACGAAGACTTTGTGTGGACA GTAATGACCTCACGTTTCCGATTGCCTGCTGGCAGAA
PTPN6	NM_002831.5	1734-1834	TGGTGCAGACGGAGGCGCAGTACAAGTTCATCTACGTGGCCATCGCCCAGTTCATTGAAACC ACTAAGAAGAAGCTGGAGGTCCTGCAGTCGCAGAAGGG
PTPRK	NM_001135648.1	4315-4415	GTGATCAACCGGATTTTTAGGATATGCAATCTAACAAGACCACAGGAAGGTTATCTGATGGTG CAACAGTTTCAGTACCTAGGATGGGCTTCTCATCGAG
RAB31	NM_006868.3	3800-3900	TTTTGTAAAGAGCTTCCATCTGGGCTGGACCCAGTTCTTGCACATACAAGACACCGCTGCAGT CAGCTAGGACCTTTCCGCCATGTATTCTATTCTGTAG

RAC1	NM_198829.1	1250-1350	AAAGACCTTCGTCTTTGAGAAGACGGTAGCTTCTGCAGTTAGGAGGTGCAGACACTTGCTCTC CTATGTAGTTCTCAGATGCGTAAAGCAGAACAGCCTC
RAC2	NM_002872.3	1069-1169	GCTGCCACAACTTGTGTACCTTCAGGGATGGGGCTCTTACTCCCTCCTGAGGCCAGCTGCTCT AATATCGATGGTCCTGCTTGCCAGAGAGTTCCTCTAC
RAF1	NM_002880.2	1990-2090	CCTATGGCATCGTATTGTATGAACTGATGACGGGGGAGCTTCCTTATTCTCACATCAACAACC GAGATCAGATCATCTTCATGGTGGGCCGAGGATATGC
RAP1GAP2	NM_015085.4	4140-4240	CCCACGGCTGGAAAGAGGCCTGTACGTTCTGGACGCGTTTTGTTGGCTGGGCTTCTGGAGGC ACTGGCAAGGTCAAACTGCATTTCTTTAAGAACAGTTG
RARA	NM_000964.2	115-215	AGCCACCTAGCTGGGGCCCATCTAGGAGTGGCATCTTTTTTGGTGCCCTGAAGGCCAGCTCT GGACCTTCCCAGGAAAAGTGCCAGCTCACAGAACTGCT
RBPMS	NM_001008710.1	842-942	AAACAGCCTGTAGGTTTTGTCAGTTTTGACAGTCGCTCAGAAGCAGAGGCTGCAAAGAATGCT TTGAATGGCATCCGCTTCGATCCTGAAATTCCGCAAA
RHOA	NM_001664.2	1230-1330	GGTACTCTGGTGAGTCACCACTTCAGGGCTTTACTCCGTAACAGATTTTGTTGGCATAGCTCT GGGGTGGGCAGTTTTTTGAAAATGGGCTCAACCAGAA
RNF125	NM_017831.3	790-890	GCAAGGTGTGTATGTCCCTTTTGTCAGAGGGAACTGTATGAAGACAGCTTGCTGGATCATTGT ATTACTCATCACAGATCGGAACGGAGGCCTGTGTTCT
RORA	NM_134261.2	1715-1815	AAAATTAACCGAGACACTTTATATGGCCCTGCACAGACCTGGAGCGCCACACACTGCACATCT TTTGGTGATCGGGGTCAGGCAAAGGAGGGGAAACAAT
RORC	NM_001001523.1	1350-1450	CTCATCAATGCCCATCGGCCAGGGCTCCAAGAGAAAAGGAAAGTAGAACAGCTGCAGTACAAT CTGGAGCTGGCCTTTCATCATCATCTCTGCAAGACTC
RPL27	NM_000988.3	23-123	GGGCCGGGTGGTTGCTGCCGAAATGGGCAAGTTCATGAAACCTGGGAAGGTGGTGCTTGTCC TGGCTGGACGCTACTCCGGACGCAAAGCTGTCATCGTG
RPS13	NM_001017.2	331-431	GCATCTTGAGAGGAACAGAAAGGATAAGGATGCTAAATTCCGTCTGATTCTAATAGAGAGCCG GATTCACCGTTTGGCTCGATATTATAAGACCAAGCGA
RUNX1	NM_001754.4	635-735	CAGCCATGAAGAACCAGGTTGCAAGATTTAATGACCTCAGGTTTGTCGGTCGAAGTGGAAGAG GGAAAAGCTTCACTCTGACCATCACTGTCTTCACAAA
RUNX2	NM_004348.3	1850-1950	GAAGCCACAGCAGTTCCCCAACTGTTTTGAATTCTAGTGGCAGAATGGATGAATCTGTTTGGC GACCATATTGAAATTCCTCAGCAGTGGCCCAGTGGTA
RUNX3	NM_004350.1	2085-2185	GTGGTCTCATAATTCCATTTGTGGAGAGAACAGGAGGGCCAGATAGATAGGTCCTAGCAGAAG GCATTGAGGTGAGGGATCATTTTGGGTCAGACATCAA
S100A4	NM_002961.2	263-363	CAGGGACAACGAGGTGGACTTCCAAGAGTACTGTGTCTTCCTGTCCTGCATCGCCATGATGTG TAACGAATTCTTTGAAGGCTTCCCAGATAAGCAGCCC
S100A6	NM_014624.3	539-639	TTCCTGGGGGCCTTGGCTTTGATCTACAATGAAGCCCTCAAGGGCTGAAAATAAATAGGGAAG ATGGAGACACCCTCTGGGGGTCCTCTCTGAGTCAAAT
SATB1	NM_001131010.1	1335-1435	TTCCGAAATCTACCAGTGGGTACGCGATGAACTGAAACGAGCAGGAATCTCCCAGGCGGTATT TGCACGTGTGGCTTTTAACAGAACTCAGGGCTTGCTT
SCML1	NM_001037540.1	925-1025	GCAACGTATGGTTCTTCTTCAGGGCTCTGCCTTGGCAACCCTCGGGCTGACAGCATCCACAAC ACTTACTCAACTGACCATGCTTCTGCAGCACCACCTT
SCML2	NM_006089.2	360-460	ATTGGAAGCCCGTGACCCTCGCAATGCCACTTCAGTATGTATTGCTACGGTTATTGGAATTACT GGGGCCAGGTTACGGTTACGACTGGATGGTAGTGAC
SEL1L	NM_005065.4	980-1080	GGGCAATCTAATAGCCCACATGGTTTTGGGTTACAGATACTGGGCTGGCATCGGCGTCCTCCA GAGTTGTGAATCTGCCCTGACTCACTATCGTCTTGTT
SELL	NM_000655.3	110-210	CTCCCTTTGGGCAAGGACCTGAGACCCTTGTGCTAAGTCAAGAGGCTCAATGGGCTGCAGAA GAACTAGAGAAGGACCAAGCAAAGCCATGATATTTCCA
SELPLG	NM_003006.3	2297-2397	CATGGGCTGTTAGGTTGACTTCAGTTTTGCCTCTTGGACAACAGGGGGTCTTGTACATCCTTG GGTGACCAGGAAAAGTTCAGGCTATGGGGGGCCAAAG
SERPINE2	NM_006216.2	240-340	CGCTGCCTTCCATCTGCTCCCACTTCAATCCTCTGTCTCTCGAGGAACTAGGCTCCAACACGG GGATCCAGGTTTTCAATCAGATTGTGAAGTCGAGGCC
SH2B3	NM_005475.2	4285-4385	CCTCCAGCCAGAAGTTAAACATCTGGGATATGACGTCTTCATGCCAGGGGCACTCATTTCTTA GCAGCCTCTCTACATACATCTCTCAGGTGGTGCCAAG
SH2D2A	NM_001161443.1	341-441	TGCTGGAGCCCAAGCCTCAGGGGTGCTACTTGGTGCGGTTCAGCGAGAGCGCGGTGACCTT CGTGCTGACTTACAGGAGCCGGACTTGCTGCCGCCACTT
SIT1	NM_014450.2	720-820	GCCCCAGCCCCCCGTAGCAGGGGCATGACTGTTTCCCAACCAGCACCCAAAGACGGGCGCC ATTGCCAAGTCACAGGATGTGATCTACCCCGGACTTCCT
SKAP1	NM_003726.3	1360-1460	AAGTGGGAAGAGGCACGTTCATCAAACCTGTTACTAAACCAGCCTAGTCATAGCTCATCCCCA TCTCTAAATGTGTCCACACAACCACATCTGCCTTTTC
SKAP2	NM_003930.3	3374-3474	TTTTACAGTTAATCCAGGAGAGGGAGTCCTTTGCCAACTGATGACCAACAGTTCCAAGCCAGA TAGTCTCGTGAACAGTGACAATACAGAAATAAGGTGT
SLA2	NM_032214.2	1640-1740	AAAGGAAAGCTGAGATGATGTCTTACCGTAGCAGCAGATCTTGGATGGTCCAGGCTCTATGTG ACCTCCAGAGCAAAGAGAAAGACTTCGGACAGTCTAG
SLAMF1	NM_003037.2	580-680	GTGTCTCTTGATCCATCCGAAGCAGGCCCTCCACGTTATCTAGGAGATCGCTACAAGTTTTATC TGGAGAATCTCACCCTGGGGATACGGGAAAGCAGGA
SLAMF7	NM_021181.3	215-315	GGGCACTATCATAGTGACCCAAAATCGTAATAGGGAGAGAGTAGACTTCCCAGATGGAGGCTA CTCCCTGAAGCTCAGCAAACTGAAGAAGAATGACTCA
SLC2A1	NM_006516.2	2500-2600	AGGCTCCATTAGGATTTGCCCCTTCCCATCTCTTCCTACCCAACCACTCAAATTAATCTTTCTTT ACCTGAGACCAGTTGGGAGCACTGGAGTGCAGGGA
SMAD3	NM_005902.3	4220-4320	TTAAAGGACAGTTGAAAAGGGCAAGAGGAAACCAGGGCAGTTCTAGAGGAGTGCTGGTGACT GGATAGCAGTTTTAAGTGGCGTTCACCTAGTCAACACG
SMAD4	NM_005359.3	1370-1470	AGGTTGCACATAGGCAAAGGTGTGCAGTTGGAATGTAAAGGTGAAGGTGATGTTTGGGTCAG GTGCCTTAGTGACCACGCGGTCTTTGTACAGAGTTACT

SNAI1	NM_005985.2	63-163	GACCACTATGCCGCGCTCTTTCCTCGTCAGGAAGCCCTCCGACCCCAATCGGAAGCCTAACTA CAGCGAGCTGCAGGACTCTAATCCAGAGTTTACCTTC
SOCS1	NM_003745.1	1025-1125	TTAACTGTATCTGGAGCCAGGACCTGAACTCGCACCTCCTACCTCTTCATGTTTACATATACCC AGTATCTTTGCACAAACCAGGGGTTGGGGGAGGGTC
SOCS3	NM_003955.3	1870-1970	GGAGGATGGAGGAGACGGGACATCTTTCACCTCAGGCTCCTGGTAGAGAAGACAGGGGATTC TACTCTGTGCCTCCTGACTATGTCTGGCTAAGAGATTC
SOD1	NM_000454.4	35-135	GCCTATAAAGTAGTCGCGGAGACGGGGTGCTGGTTTGCGTCGTAGTCTCCTGCAGCGTCTGG GGTTTCCGTTGCAGTCCTCGGAACCAGGACCTCGGCGT
SOX13	NM_005686.2	3039-3139	ATTTATTGAGTGCCCACTACGTGCCAGGCACTGTTGCTGAGTTCCTGTGGGTGTGTCTCTCGA TGCCACTCCTGCTTCTCTGGGGGCCTCTTTCTGTGCT
SOX2	NM_003106.2	151-251	CTTAAGCCTTTCCAAAAAATAATAATAACAATCATCGGCGGCGGCAGGATCGGCCAGAGGAGG AGGGAAGCGCTTTTTTTGATCCTGATTCCAGTTTGCC
SOX4	NM_003107.2	3040-3140	GTTCACGGTCAAACTGAAATGGATTTGCACGTTGGGGAGCTGGCGGCGGCGGCTGCTGGGC CTCCGCCTTCTTTTCTACGTGAAATCAGTGAGGTGAGAC
SOX5	NM_152989.2	1885-1985	TAGCCATGCAATGATGGATTTCAATCTGAGTGGAGATTCTGATGGAAGTGCTGGAGTCTCAGA GTCAAGAATTTATAGGGAATCCCGAGGGCGTGGTAGC
SPI1	NM_003120.1	730-830	CTCCGCAGCGGCGACATGAAGGACAGCATCTGGTGGGTGGACAAGGACAAGGGCACCTTCC AGTTCTCGTCCAAGCACAAGGAGGCGCTGGCGCACCGCT
SPN	NM_001030288.1	2798-2898	AAGCCAGGCTTCATGGAAAGATCGTATGTGTGACCCAAATATGAGTTCTTCAGCTCAGCCATG GTAATCCCTTCCTTGAAGTCTCCATTTCTGCAGTACA
SPRY2	NM_005842.2	85-185	AAAGAGGAAATACTCCGCGTGCGCTTGTAGAAGGGGAGTCGTCTCCAGCTCCGAACCCCGGA GTGTTCATCAGCGGGGAATCTGGCTCCGAATTCTCTTT
STAT1	NM_007315.2	205-305	TTTGCTGTATGCCATCCTCGAGAGCTGTCTAGGTTAACGTTCGCACTCTGTGTATATAACCTCG ACAGTCTTGGCACCTAACGTGCTGTGCGTAGCTGCT
STAT3	NM_139276.2	4535-4635	AGACTTGGGCTTACCATTGGGTTTAAATCATAGGGACCTAGGGCGAGGGTTCAGGGCTTCTCT GGAGCAGATATTGTCAAGTTCATGGCCTTAGGTAGCA
STAT4	NM_003151.2	789-889	AGACAATGGATCAGAGTGACAAGAATAGTGCCATGGTGAATCAGGAAGTTTTGACACTGCAGG AAATGCTTAACAGCCTCGATTTCAAGAGAAAGGAGGC
STAT5A	NM_003152.2	3460-3560	GAGACAGAGAGAGAGAAAGAGAGAGTGTGTGGGTCTATGTAAATGCATCTGTCCTCATGTGTT GATGTAACCGATTCATCTCTCAGAAGGGAGGCTGGGG
STAT5B	NM_012448.3	200-300	AAGGAGAAGCCCTTCATCAGATGCAAGCGTTATATGGCCAGCATTTTCCCATTGAGGTGCGGC ATTATTTATCCCAGTGGATTGAAAGCCAAGCATGGGA
STAT6	NM_003153.3	2030-2130	AGAACATCCAGCCATTCTCTGCCAAAGACCTGTCCATTCGCTCACTGGGGGACCGAATCCGG GATCTTGCTCAGCTCAAAAATCTCTATCCCAAGAAGCC
STMN1	NM_203401.1	287-387	CGTGGGTGGCGGCAGGACTTTCCTTATCCCAGTTGATTGTGCAGAATACACTGCCTGTCGCTT GTCTTCTATTCACCATGGCTTCTTCTGATATCCAGGT
SYK	NM_003177.3	1685-1785	CGGACTCTCCAAAGCACTGCGTGCTGATGAAAACTACTACAAGGCCCAGACCCATGGAAAGT GGCCTGTCAAGTGGTACGCTCCGGAATGCATCAACTAC
TAL1	NM_003189.2	4635-4735	ACAGCATCTGTAGTCAGCCGACAACTATTTCGGCCTTTTGGGGGTGGGTCTGGCCGTACTTGT GATTTCGATGGTACGTGACCCTCTGCTGAAGACTTGC
TBP	NM_003194.3	25-125	CGCCGGCTGTTTAACTTCGCTTCCGCTGGCCCATAGTGATCTTTGCAGTGACCCAGCAGCATC ACTGTTTCTTGGCGTGTGAAGATAACCCAAGGAATTG
TBX21	NM_013351.1	890-990	ACACAGGAGCGCACTGGATGCGCCAGGAAGTTTCATTTGGGAAACTAAAGCTCACAAACAACA AGGGGGCGTCCAACAATGTGACCCAGATGATTGTGCT
TBXA2R	NM_001060.3	385-485	CACACGCGCTCCTCCTTCCTCACCTTCCTCTGCGGCCTCGTCCTCACCGACTTCCTGGGGCT GCTGGTGACCGGTACCATCGTGGTGTCCCAGCACGCCG
TCF12	NM_207037.1	1105-1205	CACATGACCGCTTGAGTTATCCTCCACACTCAGTTTCACCAACAGACATAAACACGAGTCTTCC ACCAATGTCCAGCTTTCATCGCGGCAGTACCAGCAG
TCF3	NM_003200.2	4325-4425	ATACGTGTCAACACAGCTGGCTGGATGATTGGGACTTTAAAACGACCCTCTTTCAGGTGGATT CAGAGACCTGTCCTGTATATAACAGCACTGTAGCAAT
TCF7	NM_003202.2	2420-2520	ATTCCATTTCCAGTTCATCTATGGCAGTCCAGCCAGCTCCTGGGCAGCTTGAGAGGGCAAACC CAAAACCTCATGACAGCCAGAGCCTGTCTTTCAGCAT
TDGF1	NM_003212.2	1567-1667	AAGGAAAGAAAACATCTTTAAGGGGAGGAACCAGAGTGCTGAAGGAATGGAAGTCCATCTGC GTGTGTGCAGGGAGACTGGGTAGGAAAGAGGAAGCAAA
TDO2	NM_005651.1	0-100	AAGGTCAATGATAGCATCTGCCTAGAGTCAAACCTCCGTGCTTCTCAGACAGTGCCTTTTCAC CATGAGTGGGTGCCCATTTTTAGGAAACAACTTTGGA
TEK	NM_000459.2	615-715	CGAGTTCGAGGAGAGGCAATCAGGATACGAACCATGAAGATGCGTCAACAAGCTTCCTTCCTA CCAGCTACTTTAACTATGACTGTGGACAAGGGAGATA
TERF1	NM_003218.3	1037-1137	CTGAAAGCAGAATACCTGTTTCAAAGAGTCAGCCGGTAACTCCTGAAAAACATCGAGCTAGAA AAAGACAGGCATGGCTTTGGGAAGAAGACAAGAATTT
TERT	NM_198253.1	2570-2670	GGCTTCAAGGCTGGGAGGAACATGCGTCGCAAACTCTTTGGGGTCTTGCGGCTGAAGTGTCA CAGCCTGTTTCTGGATTTGCAGGTGAACAGCCTCCAGA
TF	NM_001063.2	640-740	CTGCTCCACCCTTAACCAATACTTCGGCTACTCGGGAGCCTTCAAGTGTCTGAAGGATGGTGC TGGGGATGTGGCCTTTGTCAAGCACTCGACTATATTT
TFRC	NM_003234.1	1220-1320	CAGTTTCCACCATCTCGGTCATCAGGATTGCCTAATATACCTGTCCAGACAATCTCCAGAGCTG CTGCAGAAAAGCTGTTTGGGAATATGGAAGGAGACT
TGFA	NM_003236.2	780-880	TGCCACAGACCTTCCTACTTGGCCTGTAATCACCTGTGCAGCCTTTTGTGGGCCTTCAAAACT CTGTCAAGAACTCCGTCTGCTTGGGGTTATTCAGTGT
TGFB1	NM_000660.3	1260-1360	TATATGTTCTTCAACACATCAGAGCTCCGAGAAGCGGTACCTGAACCCGTGTTGCTCTCCCGG GCAGAGCTGCGTCTGCTGAGGCTCAAGTTAAAAGTGG

TGFB2	NM_003238.2	1125-1225	AAGCCAGAGTGCCTGAACAACGGATTGAGCTATATCAGATTCTCAAGTCCAAAGATTTAACATC TCCAACCCAGCGCTACATCGACAGCAAAGTTGTGAA
TGFBR1	NM_004612.2	4280-4380	GGGGAAATACGACTTAGTGAGGCATAGACATCCCTGGTCCATCCTTTCTGTCTCCAGCTGTTT CTTGGAACCTGCTCTCCTGCTTGCTGGTCCCTGACGC
Thymidine Kinase	SCFV007.1	100-200	TCTACGTACCCGAGCCGATGACTTACTGGCAGGTGCTGGGGGCTTCCGAGACAATCGCGAAC ATCTACACCACACAACACCGCCTCGACCAGGGTGAGAT
TIE1	NM_005424.2	2610-2710	CATCGGGGAGGGGAACTTCGGCCAGGTCATCCGGGCCATGATCAAGAAGGACGGGCTGAAG ATGAACGCAGCCATCAAAATGCTGAAAGAGTATGCCTCT
TLR2	NM_003264.3	180-280	CTGCTTTCAACTGGTAGTTGTGGGTTGAAGCACTGGACAATGCCACATACTTTGTGGATGGTG TGGGTCTTGGGGGTCATCATCAGCCTCTCCAAGGAAG
TLR8	NM_138636.3	2795-2895	GACAAAAACGTTCTCCTTTGTCTAGAGGAGAGGGATTGGGATCCGGGATTGGCCATCATCGAC AACCTCATGCAGAGCATCAACCAAAGCAAGAAAACAG
TNF	NM_000594.2	1010-1110	AGCAACAAGACCACCACTTCGAAACCTGGGATTCAGGAATGTGTGGCCTGCACAGTGAAGTG CTGGCAACCACTAAGAATTCAAACTGGGGCCTCCAGAA
TNFRSF14	NM_003820.2	916-1016	CTCAGGGAGCCTCGTCATCGTCATTGTTTGCTCCACAGTTGGCCTAATCATATGTGTGAAAAG AAGAAAGCCAAGGGGTGATGTAGTCAAGGTGATCGTC
TNFRSF18	NM_004195.2	445-545	AGGGGAAATTCAGTTTTGGCTTCCAGTGTATCGACTGTGCCTCGGGGACCTTCTCCGGGGGC CACGAAGGCCACTGCAAACCTTGGACAGACTGCACCCA
TNFRSF1B	NM_001066.2	835-935	CCCAGCTGAAGGGAGCACTGGCGACTTCGCTCTTCCAGTTGGACTGATTGTGGGTGTGACAG CCTTGGGTCTACTAATAATAGGAGTGGTGAACTGTGTC
TNFRSF4	NM_003327.2	200-300	CCGTGCGGGCCGGGCTTCTACAACGACGTGGTCAGCTCCAAGCCGTGCAAGCCCTGCACGT GGTGTAACCTCAGAAGTGGGAGTGAGCGGAAGCAGCTGT
TNFRSF9	NM_001561.4	255-355	AGATTTGCAGTCCCTGTCCTCCAAATAGTTTCTCCAGCGCAGGTGGACAAAGGACCTGTGACA TATGCAGGCAGTGTAAAGGTGTTTTCAGGACCAGGAA
TNFSF10	NM_003810.2	115-215	GGGGGGACCCAGCCTGGGACAGACCTGCGTGCTGATCGTGATCTTCACAGTGCTCCTGCAGT CTCTCTGTGTGGCTGTAACTTACGTGTACTTTACCAAC
TNFSF11	NM_003701.2	490-590	TACCTGATTCATGTAGGAGAATTAAACAGGCCTTTCAAGGAGCTGTGCAAAAGGAATTACAACA TATCGTTGGATCACAGCACATCAGAGCAGAGAAAGC
TNFSF14	NM_003807.2	270-370	ATTTTCAGAAGCCTCTGGAAAGTCGTGCACAGCCCAGGAGTGTTGAGCAATTTCGGTTTCCTC TGAGGTTGAAGGACCCAGGCGTGTCAGCCCTGCTCCA
TOX	NM_014729.2	3950-4050	AATGAGCAGCTTTGACTTTGACAGGCGGTTTGTGCAGGAAAGCACAGTGCCGTGTTGTTTACA GCTTTTCTAGAGCAGCTGTGCGACCAGGGTAGAGAGT
TP53	NM_000546.2	1330-1430	GGGGAGCAGGGCTCACTCCAGCCACCTGAAGTCCAAAAAGGGTCAGTCTACCTCCCGCCATA AAAAACTCATGTTCAAGACAGAAGGGCCTGACTCAGAC
TRAF1	NM_005658.3	3735-3835	CGAGTGATGGGTCTAGGCCCTGAAACTGATGTCCTAGCAATAACCTCTTGATCCCTACTCACC GAGTGTTGAGCCCAAGGGGGGATTTGTAGAACAAGCC
TRAF2	NM_021138.3	1325-1425	GTGGCCCTTCAACCAGAAGGTGACCTTAATGCTGCTCGACCAGAATAACCGGGAGCACGTGA TTGACGCCTTCAGGCCCGACGTGACTTCATCCTCTTTT
TRAF3	NM_145725.1	1795-1895	ATATGATGCCCTGCTTCCTTGGCCGTTTAAGCAGAAAGTGACACTCATGCTGATGGATCAGGG GTCCTCTCGACGTCATTTGGGAGATGCATTCAAGCCC
TSC22D3	NM_198057.2	1400-1500	TTAAGCAGAGGCAACCTCTCTCTTCTCCTCTGTTTCGTGAAGGCAGGGGACACAGATGGGAGA GATTGAGCCAAGTCAGCCTTCTGTTGGTTAATATGGT
TSLP	NM_033035.3	395-495	CCGTCTCTTGTAGCAATCGGCCACATTGCCTTACTGAAATCCAGAGCCTAACCTTCAATCCCAC CGCCGGCTGCGCGTCGCTCGCCAAAGAAATGTTCGC
TXK	NM_003328.1	800-900	ATGACTCGTCTCCGATATCCAGTTGGGCTGATGGGCAGTTGTTTACCAGCCACAGCTGGGTTT AGCTACGAAAAGTGGGAGATAGATCCATCTGAGTTGG
TYK2	NM_003331.3	485-585	TCATCGCTGACAGCTGAGGAAGTCTGCATCCACATTGCACATAAAGTTGGTATCACTCCTCCTT GCTTCAATCTCTTTGCCCTCTTCGATGCTCAGGCCC
TYROBP	NM_003332.2	457-557	CTGCACCTCATTCCAACTCCTACCGCGATACAGACCCACAGAGTGCCATCCCTGAGAGACCAG ACCGCTCCCCAATACTCTCCTAAAATAAACATGAAGC
UBASH3A	NM_001001895.1	1970-2070	GAGATGCTGCTGTTTCCAGAGGCGTCTTAGTCTCACCCAATGTGATTTGTAGAAGCACGAGAC GCACTTTTATATCCCGGAATATTTCCCTCCGGCTTTC
VAX2	NM_012476.2	871-971	CAGCGCCAGCAGCTGCAAGAAAGCTAACACTTAAGACTCCCACCCTGTGACACTGAGTCCCG AGCACAGCACCTTCCCAGTCTCCTGTGCCCCAGCGGAC
VEGFA	NM_001025366.1	1325-1425	GAGTCCAACATCACCATGCAGATTATGCGGATCAAACCTCACCAAGGCCAGCACATAGGAGAG ATGAGCTTCCTACAGCACAACAAATGTGAATGCAGAC
WEE1	NM_003390.2	5-105	TGCGTTTGAGTTTGCCGCGAGCCGGGCCAATCGGTTTTGCCAACGCATGCCCACGTGCTGGC GAACAAATGTAAACACGGAGATCGTGTGCCGGGCACTT
XBP1	NM_005080.2	440-540	GGAGTTAAGACAGCGCTTGGGGATGGATGCCCTGGTTGCTGAAGAGGAGGCGGAAGCCAAG GGGAATGAAGTGAGGCCAGTGGCCGGGTCTGCTGAGTCC
XBP1	NM_001079539.1	935-1035	ATTCATTGTCTCAGTGAAGGAAGAACCTGTAGAAGATGACCTCGTTCCGGAGCTGGGTATCTC AAATCTGCTTTCATCCAGCCACTGCCCAAAGCCATCT
YY1AP1	NM_139118.2	755-855	ATGGGAGCTATGCAGCTGATTGAAGACTTCAGCACACATGTCAGCATTGACTGCAGCCCTCAT AAAACTGTCAAGAAGACTGCCAATGAATTTCCCTGTT
ZAP70	NM_001079.3	1175-1275	GGAGCTCAAGGACAAGAAGCTCTTCCTGAAGCGCGATAACCTCCTCATAGCTGACATTGAACT TGGCTGCGGCAACTTTGGCTCAGTGCGCCAGGGCGTG
ZBTB16	NM_006006.4	1585-1685	TCCTGGATAGTTTGCGGCTGAGAATGCACTTACTGGCTCATTCAGCGGGTGCCAAAGCCTTTG TCTGTGATCAGTGCGGTGCACAGTTTTCGAAGGAGGA
ZC2HC1A	NM_016010.2	665-765	ACGATTACCGCAGCCAAGTGGCGCTGGCAAAACTGTTGTAGGTGTTCCTTCAGGTAAAGTGTC TTCAAGTAGCAGCTCTTTGGGAAACAAACTTCAGACC

ZEB2	NM_014795.2	20-120	TCCCAGAGAGAAACTTGGCGATCACGTTTTCACATGATGCTCACGCTCAGGGCGCTTCAATTA TCCCTCCCCACAAAGATAGGTGGCGCGTGTTTCAGGG
ZNF516	NM_014643.2	4830-4930	GGTGGGGGACGGCTTCATATACCTCTTCCTCAGTAATGCAAATGCGAGTTTTTGTGGTGGGGG TTAAGGCCCATAACAAAGGATCTTAAACCATGCAGTG

Appendix B. Fold change in mRNA abundance of mIL15-CAR T cells relative to CAR T cells

GENE	logFC	PValue	FDR
IL7RA	-2.4	1.9E-05	6.7E-03
NRP1	-2.7	1.6E-04	2.9E-02
DAP12	1.9	3.1E-04	3.7E-02
CD4	-1.9	5.5E-04	4.9E-02
TNFRSF4	-1.7	2.5E-03	1.5E-01
TIE1	-1.9	2.6E-03	1.5E-01
HRH2	1.6	4.6E-03	2.2E-01
CPT1	-1.6	5.0E-03	2.2E-01
PDCD1	-1.6	8.6E-03	3.4E-01
ICOSLG	-1.3	1.3E-02	4.2E-01
CMRF35H	-1.7	1.4E-02	4.2E-01
CCR4	-1.4	1.4E-02	4.2E-01
RORC	1.4	2.5E-02	6.4E-01
GILZ	-1.1	2.6E-02	6.4E-01
CD86	-1.3	2.7E-02	6.4E-01
TSLPR	-1.5	3.1E-02	6.8E-01
BCL6	-1.1	3.8E-02	7.9E-01
PAX5	1.1	4.2E-02	8.3E-01
IL23R	1.1	4.8E-02	8.9E-01
KIR2DS2	1.1	5.2E-02	9.2E-01
CD40LG	-1.1	6.3E-02	1.0E+00
IKZF2	-1.0	7.6E-02	1.0E+00
BCL2	0.8	9.2E-02	1.0E+00
SOX5	1.3	1.1E-01	1.0E+00
SOX13	-0.9	1.4E-01	1.0E+00
BNIP3	-0.8	1.5E-01	1.0E+00
GZMH	0.7	1.5E-01	1.0E+00
CD11B	-0.8	1.6E-01	1.0E+00
LEF1	0.9	1.6E-01	1.0E+00
SMAD3	-0.7	1.6E-01	1.0E+00
FLT1	0.7	1.6E-01	1.0E+00
KLRB1	-0.7	1.7E-01	1.0E+00
CCL5	-0.6	1.7E-01	1.0E+00
C5ORF13	-0.8	1.8E-01	1.0E+00
KLF2	-0.6	2.0E-01	1.0E+00
CD276	-0.9	2.0E-01	1.0E+00
LRP5	0.7	2.0E-01	1.0E+00
P2RX7	-0.7	2.1E-01	1.0E+00
NCAM1	0.6	2.1E-01	1.0E+00
ATF3	0.8	2.2E-01	1.0E+00
ANXA1	-0.7	2.2E-01	1.0E+00
EOMES	-0.8	2.3E-01	1.0E+00
NFATC1	-0.6	2.3E-01	1.0E+00
MMP14	-0.8	2.5E-01	1.0E+00
PHACTR2	-0.7	2.6E-01	1.0E+00
GATA3	-0.7	2.6E-01	1.0E+00
CD44	-0.6	2.6E-01	1.0E+00
AIM2	-0.6	2.7E-01	1.0E+00
AGER	-0.6	2.7E-01	1.0E+00
TGFBR1	0.6	2.8E-01	1.0E+00
CSF2	-0.7	2.9E-01	1.0E+00
CD80	-0.6	3.0E-01	1.0E+00
IL10	0.4	3.2E-01	1.0E+00
FGL2	-0.6	3.3E-01	1.0E+00
MYC	-0.5	3.4E-01	1.0E+00
PHC1	-0.6	3.5E-01	1.0E+00
LOC282997	-0.5	3.5E-01	1.0E+00
ITGA5	-0.6	3.6E-01	1.0E+00
SIP1	-0.5	3.7E-01	1.0E+00
ITGA1	-0.5	3.7E-01	1.0E+00
KIR3DL2	-0.8	3.7E-01	1.0E+00
TBXA2R	0.6	3.8E-01	1.0E+00
CCR7	-0.5	3.9E-01	1.0E+00
RNF125	-0.5	3.9E-01	1.0E+00
ITGA4	-0.4	4.1E-01	1.0E+00
IL17RA	0.4	4.1E-01	1.0E+00
NOTCH1	0.4	4.1E-01	1.0E+00
CD28	-0.4	4.2E-01	1.0E+00
BIM	-0.5	4.2E-01	1.0E+00
STMN1	-0.4	4.3E-01	1.0E+00
LYN	0.4	4.3E-01	1.0E+00
KIR2DS1	0.4	4.3E-01	1.0E+00
NKp30	0.4	4.4E-01	1.0E+00
TNFRSF18	-0.5	4.4E-01	1.0E+00
RUNX1	0.4	4.4E-01	1.0E+00
SH2D2A	0.4	4.5E-01	1.0E+00
PDK1	-0.4	4.5E-01	1.0E+00
SELL	-0.4	4.6E-01	1.0E+00
POU5F1	-0.5	4.7E-01	1.0E+00
EGLN1	-0.4	4.7E-01	1.0E+00
TRAF1	-0.4	4.7E-01	1.0E+00
SOCS1	0.4	4.7E-01	1.0E+00
KIR2DL1	-0.7	4.8E-01	1.0E+00
IL23p19	0.4	4.8E-01	1.0E+00
LNK	0.3	4.9E-01	1.0E+00
SKAP1	-0.4	4.9E-01	1.0E+00
BIRC2	-0.3	4.9E-01	1.0E+00
STAT1	-0.2	5.0E-01	1.0E+00
LAIR1	-0.3	5.0E-01	1.0E+00
TNFRSF9	-0.5	5.0E-01	1.0E+00
MYB	-0.4	5.0E-01	1.0E+00
CDK4	0.4	5.0E-01	1.0E+00
XBP1	0.4	5.0E-01	1.0E+00
TFRC	-0.4	5.0E-01	1.0E+00
SLA2	0.4	5.1E-01	1.0E+00
CD69	0.3	5.1E-01	1.0E+00
FAS	-0.4	5.1E-01	1.0E+00
CCL3	0.2	5.1E-01	1.0E+00
EGLN3	-0.3	5.1E-01	1.0E+00
TNF	0.2	5.2E-01	1.0E+00
IGF1R	-0.3	5.2E-01	1.0E+00

BACH2	0.3	5.2E-01	1.0E+00
CD94	-0.4	5.2E-01	1.0E+00
BCL2L1	0.3	5.2E-01	1.0E+00
TXK	0.3	5.2E-01	1.0E+00
IL10RA	-0.3	5.3E-01	1.0E+00
RARA	-0.4	5.3E-01	1.0E+00
XBP1	0.3	5.3E-01	1.0E+00
IL12RB2	0.4	5.3E-01	1.0E+00
DPP4	0.3	5.3E-01	1.0E+00
RUNX3	0.4	5.4E-01	1.0E+00
HVEM	-0.3	5.4E-01	1.0E+00
FLT3LG	-0.3	5.4E-01	1.0E+00
ITGAL	-0.3	5.5E-01	1.0E+00
EPHA4	-0.3	5.5E-01	1.0E+00
IL2RA	-0.4	5.5E-01	1.0E+00
PTGER2	-0.3	5.6E-01	1.0E+00
ID2	0.3	5.6E-01	1.0E+00
DNAM1	-0.2	5.7E-01	1.0E+00
CD7	0.3	5.7E-01	1.0E+00
SLAMF1	-0.4	5.7E-01	1.0E+00
BCLXL	0.2	5.9E-01	1.0E+00
SATB1	0.3	5.9E-01	1.0E+00
TP53	0.2	5.9E-01	1.0E+00
MAD1L1	0.3	6.0E-01	1.0E+00
FOS	0.3	6.0E-01	1.0E+00
NKG2D	-0.3	6.0E-01	1.0E+00
CDK2	0.2	6.0E-01	1.0E+00
GZMB	0.2	6.0E-01	1.0E+00
IFNGR1	0.4	6.0E-01	1.0E+00
CTNNA1	0.2	6.1E-01	1.0E+00
ICOS	-0.2	6.2E-01	1.0E+00
SNAI1	-0.3	6.2E-01	1.0E+00
KIR2DL3	0.2	6.2E-01	1.0E+00
LAG3	0.2	6.2E-01	1.0E+00
HES1	-0.3	6.2E-01	1.0E+00
CD85	-0.3	6.3E-01	1.0E+00
PRF1	0.2	6.4E-01	1.0E+00
CDH1	-0.3	6.5E-01	1.0E+00
VEGFA	-0.2	6.5E-01	1.0E+00
mTOR	0.3	6.6E-01	1.0E+00
AKT1	0.2	6.6E-01	1.0E+00
CD38	-0.2	6.6E-01	1.0E+00
TRAF3	0.3	6.6E-01	1.0E+00
GLIPR1	-0.2	6.6E-01	1.0E+00
Dock5	0.2	6.6E-01	1.0E+00
BCL2L11	-0.3	6.6E-01	1.0E+00
LAT2	-0.3	6.6E-01	1.0E+00
ANXA2P2	0.2	6.7E-01	1.0E+00
p38	0.3	6.7E-01	1.0E+00
ETV6	-0.2	6.7E-01	1.0E+00
ATM	0.3	6.7E-01	1.0E+00
KIR3DL3	0.3	6.8E-01	1.0E+00
CBLB	-0.3	6.8E-01	1.0E+00
IL15RA	-0.2	6.9E-01	1.0E+00
CATHEPSINC	0.2	6.9E-01	1.0E+00
MAPK8	-0.2	6.9E-01	1.0E+00
FYN	-0.2	6.9E-01	1.0E+00
ITGB1	-0.3	6.9E-01	1.0E+00
RUNX2	-0.2	7.0E-01	1.0E+00
CCR1	0.1	7.0E-01	1.0E+00
PTPN4	-0.2	7.0E-01	1.0E+00
FASLG	0.2	7.0E-01	1.0E+00
CD19Rscfv	-0.3	7.0E-01	1.0E+00
PDE4	-0.2	7.1E-01	1.0E+00
STAT5A	-0.2	7.1E-01	1.0E+00
SHP2	0.2	7.1E-01	1.0E+00
E2A	-0.2	7.2E-01	1.0E+00
CREM	0.2	7.2E-01	1.0E+00
LTA	-0.3	7.3E-01	1.0E+00
FOXO3	-0.2	7.3E-01	1.0E+00
CASP1	0.2	7.3E-01	1.0E+00
SHP1	0.2	7.3E-01	1.0E+00
BATF	0.2	7.4E-01	1.0E+00
MAD	-0.1	7.4E-01	1.0E+00
SOCS3	-0.2	7.4E-01	1.0E+00
NKG2F	-0.3	7.4E-01	1.0E+00
IRF4	-0.2	7.4E-01	1.0E+00
RAC2	0.2	7.4E-01	1.0E+00
NFATC2	0.2	7.5E-01	1.0E+00
STAT6	-0.2	7.5E-01	1.0E+00
GNLY	0.2	7.5E-01	1.0E+00
CCL4	0.0	7.5E-01	1.0E+00
CD11C	-0.2	7.5E-01	1.0E+00
CSAD	-0.2	7.5E-01	1.0E+00
ABCB1	-0.2	7.6E-01	1.0E+00
CD8A	0.2	7.6E-01	1.0E+00
BMI1	-0.1	7.6E-01	1.0E+00
RAC1	0.2	7.6E-01	1.0E+00
LAT	-0.1	7.6E-01	1.0E+00
PNK	-0.2	7.6E-01	1.0E+00
AP1	0.2	7.6E-01	1.0E+00
STAT5B	-0.2	7.6E-01	1.0E+00
CD19RCD28CAR	-0.3	7.7E-01	1.0E+00
CITED2	0.2	7.7E-01	1.0E+00
DGKA	-0.2	7.7E-01	1.0E+00
CD274	-0.2	7.7E-01	1.0E+00
YAP	-0.1	7.7E-01	1.0E+00
IL2RG	-0.2	7.7E-01	1.0E+00
POP5	0.2	7.7E-01	1.0E+00
TRF	0.2	7.7E-01	1.0E+00
SLC2A1	-0.2	7.8E-01	1.0E+00
NFATC3	-0.2	7.8E-01	1.0E+00
CFLIP	-0.1	7.9E-01	1.0E+00
SERPINE2	-0.3	7.9E-01	1.0E+00
PDCD1LG2	-0.1	8.0E-01	1.0E+00
CTLA4	-0.2	8.0E-01	1.0E+00
SLAMF7	0.1	8.0E-01	1.0E+00
HOPX	0.1	8.1E-01	1.0E+00
TERT	0.2	8.1E-01	1.0E+00
NFAT5	-0.1	8.1E-01	1.0E+00
SCAP2	0.1	8.1E-01	1.0E+00
TNFRSF1B	-0.1	8.1E-01	1.0E+00

FANCC	0.1	8.1E-01	1.0E+00
JUNB	0.1	8.1E-01	1.0E+00
NT5E	-0.1	8.1E-01	1.0E+00
LGALS1	-0.2	8.1E-01	1.0E+00
ITK	0.1	8.1E-01	1.0E+00
BAX	0.2	8.1E-01	1.0E+00
CCR6	-0.1	8.2E-01	1.0E+00
APAF1	-0.2	8.2E-01	1.0E+00
BTLA	0.2	8.2E-01	1.0E+00
GADD45alpha	0.1	8.2E-01	1.0E+00
B2M	-0.1	8.2E-01	1.0E+00
LDHA	-0.2	8.2E-01	1.0E+00
CD43	-0.1	8.2E-01	1.0E+00
KLRC1	0.0	8.3E-01	1.0E+00
STS2	-0.2	8.3E-01	1.0E+00
HOXB3	-0.2	8.3E-01	1.0E+00
IL15	0.2	8.3E-01	1.0E+00
RAP46	-0.1	8.3E-01	1.0E+00
CDKN2C	0.1	8.3E-01	1.0E+00
MCL1	-0.1	8.4E-01	1.0E+00
CAT	-0.1	8.4E-01	1.0E+00
HPRT1	0.1	8.4E-01	1.0E+00
IKZF1	0.1	8.4E-01	1.0E+00
JAK2	-0.1	8.4E-01	1.0E+00
NEIL2	-0.2	8.4E-01	1.0E+00
NFkB	-0.1	8.5E-01	1.0E+00
CXCR4	0.1	8.5E-01	1.0E+00
KLF10	0.2	8.5E-01	1.0E+00
DUSP16	-0.1	8.5E-01	1.0E+00
MAP2K1	-0.1	8.6E-01	1.0E+00
IL21R	0.1	8.6E-01	1.0E+00
RAF1	0.1	8.6E-01	1.0E+00
FOXO1	0.1	8.6E-01	1.0E+00
MIF	-0.1	8.6E-01	1.0E+00
FADD	0.1	8.6E-01	1.0E+00
GADD45beta	0.2	8.6E-01	1.0E+00
FOXP1	0.1	8.7E-01	1.0E+00
HLAA	-0.1	8.7E-01	1.0E+00
NR3C1	-0.1	8.7E-01	1.0E+00
PRKCQ	0.1	8.8E-01	1.0E+00
TCF12	0.1	8.8E-01	1.0E+00
NR4A1	-0.1	8.8E-01	1.0E+00
BCL10	-0.1	8.8E-01	1.0E+00
ITGB7	0.0	8.8E-01	1.0E+00
MAPK14	0.1	8.8E-01	1.0E+00
HDAC2	0.1	8.8E-01	1.0E+00
CD244	0.0	8.9E-01	1.0E+00
IL18R1	0.1	8.9E-01	1.0E+00
S100A4	0.1	8.9E-01	1.0E+00
HOXB4	-0.1	8.9E-01	1.0E+00
CSNK2A1	0.1	8.9E-01	1.0E+00
BCL11B	0.0	9.0E-01	1.0E+00
CTNNBL1	0.1	9.0E-01	1.0E+00
NCL	0.1	9.0E-01	1.0E+00
ELF1	0.1	9.0E-01	1.0E+00
MBD2	0.1	9.0E-01	1.0E+00

DAP10	-0.1	9.0E-01	1.0E+00
ELF4	0.1	9.0E-01	1.0E+00
GSK3B	-0.1	9.1E-01	1.0E+00
IRF1	0.0	9.1E-01	1.0E+00
CDKN1A	-0.1	9.1E-01	1.0E+00
CREB1	0.0	9.1E-01	1.0E+00
TDO2	0.1	9.1E-01	1.0E+00
STAT3	-0.1	9.1E-01	1.0E+00
AHNAK	0.1	9.1E-01	1.0E+00
CD63	0.0	9.1E-01	1.0E+00
LCK	0.0	9.1E-01	1.0E+00
KLF6	0.1	9.2E-01	1.0E+00
IL2RB	0.0	9.2E-01	1.0E+00
NKp46	0.0	9.2E-01	1.0E+00
EIF1	0.1	9.2E-01	1.0E+00
TRAF2	0.1	9.2E-01	1.0E+00
DOK2	0.1	9.2E-01	1.0E+00
ADAM19	-0.1	9.2E-01	1.0E+00
GFI1	0.0	9.2E-01	1.0E+00
NKG2E	-0.1	9.3E-01	1.0E+00
AIF1	0.0	9.3E-01	1.0E+00
HMGB2	0.0	9.3E-01	1.0E+00
HDAC1	0.1	9.3E-01	1.0E+00
CTNNB1	0.0	9.3E-01	1.0E+00
TYK2	0.1	9.3E-01	1.0E+00
IAP	0.1	9.3E-01	1.0E+00
C80RF70	0.2	9.3E-01	1.0E+00
PPARA	0.0	9.3E-01	1.0E+00
PRDM1	0.0	9.4E-01	1.0E+00
ITCH	0.0	9.4E-01	1.0E+00
CLA	0.0	9.4E-01	1.0E+00
LGALS3	0.1	9.4E-01	1.0E+00
CATHEPSIND	0.0	9.4E-01	1.0E+00
IL18RAP	0.0	9.4E-01	1.0E+00
IL4R	0.0	9.5E-01	1.0E+00
TNFSF10	0.0	9.5E-01	1.0E+00
CD58	0.0	9.5E-01	1.0E+00
BID	0.1	9.5E-01	1.0E+00
JAK1	0.0	9.5E-01	1.0E+00
CD247	0.0	9.5E-01	1.0E+00
SOD1	0.0	9.6E-01	1.0E+00
CIITA	0.1	9.6E-01	1.0E+00
CRIP1	0.1	9.6E-01	1.0E+00
BetaArrestin	0.1	9.6E-01	1.0E+00
NEIL1	-0.1	9.6E-01	1.0E+00
SMAD4	0.0	9.6E-01	1.0E+00
SELL	0.0	9.6E-01	1.0E+00
C11ORF17	0.0	9.6E-01	1.0E+00
TNFSF14	0.0	9.6E-01	1.0E+00
RHOA	0.0	9.6E-01	1.0E+00
CCR5	-0.1	9.6E-01	1.0E+00
NKG2C	-0.1	9.7E-01	1.0E+00
S100A6	0.0	9.7E-01	1.0E+00
SIT1	0.0	9.7E-01	1.0E+00
KIR2DL4	-0.1	9.7E-01	1.0E+00
CD3E	0.0	9.7E-01	1.0E+00

GZMA	0.0	9.7E-01	1.0E+00
TBX21	0.0	9.7E-01	1.0E+00
GLO1	0.1	9.7E-01	1.0E+00
IFNG	-0.1	9.7E-01	1.0E+00
IL12RB1	0.0	9.7E-01	1.0E+00
CCNB1	-0.1	9.7E-01	1.0E+00
IRF2	0.0	9.7E-01	1.0E+00
PDE7	0.0	9.7E-01	1.0E+00
TNFRSF7	0.0	9.8E-01	1.0E+00
CLIC1	0.0	9.8E-01	1.0E+00
JAK3	0.0	9.8E-01	1.0E+00
OPTN	0.0	9.8E-01	1.0E+00
MAPK3	0.0	9.8E-01	1.0E+00
RORA	0.1	9.8E-01	1.0E+00
KLF7	0.0	9.9E-01	1.0E+00
CDKN1B	0.0	9.9E-01	1.0E+00

PPP2R1A	0.0	9.9E-01	1.0E+00
ATP2B4	0.0	9.9E-01	1.0E+00
CD2	0.0	9.9E-01	1.0E+00
CXCR3	0.0	9.9E-01	1.0E+00
C10RF24	0.0	9.9E-01	1.0E+00
TGFB1	0.0	9.9E-01	1.0E+00
STAT4	0.0	1.0E+00	1.0E+00
ZAP70	0.0	1.0E+00	1.0E+00
CD3D	0.0	1.0E+00	1.0E+00
GABPa	0.0	1.0E+00	1.0E+00
CCR10	-0.1	1.0E+00	1.0E+00
PECAM1	-0.1	1.0E+00	1.0E+00
SOX4	0.1	1.0E+00	1.0E+00

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VITA

Lenka Victoria Hurton was born in Victoria, British Columbia, Canada on July 13, 1978 to Alena and Stan Hurton. She graduated from Windsor Secondary High School in 1996 and went on to attend Fairfield University in Fairfield, CT on a full athletic scholarship. In 2000, she completed her Bachelor's Degree of Science in Biology along with minors in Marine Science and Environmental Science. After graduation she moved to New York to work for nearly a year at Cold Spring Harbor Laboratory as a research assistant in the proteomics lab of Dr. Ryuji Kobayashi. She then relocated to Virginia to pursue a M.Sc. degree at Virginia Polytechnic Institute and State University under the mentorship of Dr. Jim Berkson. In 2003, she obtained her M.Sc. in Fisheries and Wildlife Sciences. In 2005 she moved to Texas where she worked as a senior research assistant in the proteomics lab of Dr. Kobayashi. She enrolled in the Ph.D. program at the Graduate School of Biomedical Sciences at the University of Texas – Houston, MD Anderson Cancer Center in August 2006. In May 2007 she joined the Immunology program and began her dissertation work in the immunotherapy field under the mentorship of Dr. Laurence J.N. Cooper.