PI3Kδ coordinates transcriptional, chromatin, and metabolic changes to promote effector CD8+ T cells at the expense of central memory

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SUMMARY

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AUTHOR CONTRIBUTIONS
J.L.C., A.V.V., S.M.K., S.P., H.-Y.S., and J.G.-R. designed, performed, and interpreted experiments and edited the manuscript. J.M.R., B.H., T.W., J.S.M., H.S., Z.K., S.G., and R.H. helped perform experiments. H.C.S., D.B.M., J.J.O., P.J.M., and G.U. provided critical reagents and scientific input. J.L.C. and P.L.S. were responsible for project design and manuscript writing.

DECLARATION OF INTERESTS
S.P. is an employee of AstraZeneca and may own stock or stock options. J.G.-R. is an employee of TCR2. The remaining authors declare no competing interests.

SUPPLEMENTAL INFORMATION
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Patients with activated phosphatidylinositol 3-kinase delta (PI3Kδ) syndrome (APDS) present with sinopulmonary infections, lymphadenopathy, and cytomegalovirus (CMV) and/or Epstein-Barr virus (EBV) viremia, yet why patients fail to clear certain chronic viral infections remains incompletely understood. Using patient samples and a mouse model (Pik3cd<sup>E1020K/+</sup> mice), we demonstrate that, upon activation, Pik3cd<sup>E1020K/+</sup> CD8<sup>+</sup> T cells exhibit exaggerated features of effector populations both <em>in vitro</em> and after viral infection that are associated with increased Fas-mediated apoptosis due to sustained FoxO1 phosphorylation and Fasl derepression, enhanced mTORC1 and c-Myc signatures, metabolic perturbations, and an altered chromatin landscape. Conversely, Pik3cd<sup>E1020K/+</sup> CD8<sup>+</sup> cells fail to sustain expression of proteins critical for central memory, including TCF1. Strikingly, activated Pik3cd<sup>E1020K/+</sup> CD8<sup>+</sup> cells exhibit altered transcriptional and epigenetic circuits characterized by pronounced interleukin-2 (IL-2)/STAT5 signatures and heightened IL-2 responses that prevent differentiation to memory-like cells in IL-15. Our data position PI3Kδ as integrating multiple signaling nodes that promote CD8<sup>+</sup> T cell effector differentiation, providing insight into phenotypes of patients with APDS.

**In brief**

Using T cells from patients and a mouse model of activated PI3Kδ syndrome (APDS), Cannons et al. provide evidence that activated PI3Kδ drives transcriptional, chromatin, and metabolic changes involving IL-2, mTOR, Myc, and TCF1 that promote the differentiation of terminal and long-lived effector populations at the expense of central memory cells.

**Graphical Abstract**
INTRODUCTION

Following T cell receptor (TCR) ligation and cytokine signals, naive CD8+ T cells proliferate and differentiate into effector cytotoxic T lymphocytes (CTLs) that express cytolysic proteins and help eliminate virus-infected cells and tumors (Stinchcombe and Griffiths, 2007). When infections are resolved, the CTL population contracts; however, a fraction persists as long-lived memory cells, including central and effector memory populations (T_{CM} and T_{EM} cells) that provide protection against subsequent infection (Hashimoto et al., 2019). T_{CM} cells, in particular, expand upon rechallenge and maintain long-term immunity. Recently, it has been recognized that T_{EM} contain a subset of long-lived effector cells (LLECs) or terminally differentiated (terminal)-T_{EM} cells with potent cytolytic activity, in addition to longer-lived true T_{EM} cells that express high CD127 and CD27 (Milner et al., 2020; Renkema et al., 2020).

Many studies have enumerated factors critical for T cell memory, including cytokines, transcription factors (TFs), chromatin remodelers, and metabolic regulators. Of note, there is considerable overlap between the transcriptomes of CD8+ T_{CM} cells and TCF1+ stem-like progenitor cells that maintain long-term T cell responses in chronic infection and cancer (McLane et al., 2019). Thus, elucidating mechanisms guiding T_{CM} cell generation is important both for understanding memory in acute infection and harnessing immune responses in chronic infection. Whether there is a common theme unifying the regulation of effector/memory cell fate decisions remains unclear.
Class IA phosphoinositide 3-kinases (PI3Ks) are lipid kinases that are important for lymphocyte signaling, differentiation, survival, metabolism, and migration (Okkenhaug, 2013); p110δ is the predominant catalytic isoform in leukocytes. Upon activation by cell surface receptors, p110 generates phosphatidylinositol-3,4,5-triphosphate (PIP₃), which recruits proteins containing pleckstrin homology and other PIP₃-binding domains to the membrane (Okkenhaug, 2013). These include AKT kinases, which phosphorylate FoxO and other TFs, regulators of the mammalian target of rapamycin (mTOR) complex I, and other targets (Okkenhaug, 2013). Several of these molecules are downstream effectors of the cytokines interleukin-2 (IL-2) and IL-15, which influence T cell activation and memory, respectively.

Patients with gain-of-function mutations in PIK3CD, which encodes p110δ, exhibit a primary immunodeficiency, activated PI3Kδ syndrome (APDS), characterized by lymphopenia, lymphoproliferation, and recurrent respiratory infections (Angulo et al., 2013; Coulter et al., 2017; Lucas et al., 2014). A significant fraction of patients have persistent Epstein-Barr virus (EBV) or cytomegalovirus (CMV) viremia, suggesting defective clearance of certain chronic viral infections, despite having EBV-specific CD8⁺ T cells (Lucas et al., 2014). How activated PI3Kδ links to specific phenotypes remains only partially understood.

Here, we use a mouse model of patients with APDS (Pik3cd<sup>E1020K/+</sup> mice) (Preite et al., 2018) and patient samples to dissect how hyperactive PI3Kδ influences CD8⁺ T cell survival, differentiation, and function. We find that a substantial fraction of activated PI3Kδ T cells die via TCR-induced apoptosis (Angulo et al., 2013; Edwards et al., 2019), which we link to impaired FoxO1-mediated repression of Fasl. Surviving Pik3cd<sup>E1020K/+</sup> CD8⁺ T cells display enhanced and sustained mTOR and c-Myc activation, metabolic reprogramming, and increased expression of protein synthesis machinery consistent with an accelerated effector program. In contrast, Pik3cd<sup>E1020K/+</sup> cells fail to maintain expression of TCF1 and show a shift to an LLEC phenotype after infection. Cellular, transcriptome, and ATAC-seq analyses of Pik3cd<sup>E1020K/+</sup> CD8⁺ T cells reveal early and amplified IL-2 responses that propel cells toward an effector fate, with a loss of memory potential manifested at the level of chromatin. Our work positions PI3Kδ as a master regulator of signaling and transcription networks that determine effector/memory lineage decisions and argues that stringent regulation of PI3Kδ is required for appropriate development of memory.

RESULTS
T cells from patients with APDS and Pik3cd<sup>E1020K/+</sup> mice die prematurely via FasL-mediated apoptosis

To understand how activated PI3Kδ affects CD8⁺ T cell differentiation and function, we stimulated peripheral blood mononuclear cells (PBMCs) from healthy controls and patients with APDS with anti-CD3 plus anti-CD28. Strikingly, CD4⁺ and CD8⁺ T cells from patients with APDS exhibited high percentages of Annexin-V⁺ cells (Figure 1A; Angulo et al., 2013; Bier et al., 2019). Similar results were observed with activated T cells from Pik3cd<sup>E1020K/+</sup> mice or Pik3cd<sup>E1020K/+</sup> OT-I mice expressing a class I major histocompatibility complex (MHC)-restricted transgenic TCR specific for the ovalbumin (OVA) peptide OVA<sub>257–264</sub>.
although surviving cells also showed evidence of increased proliferation (Figures S1A–S1C). Although patients with APDS and Pik3cd<sup>E1020K/+</sup> mice exhibit reduced naive T cells (Figure S1D; Angulo et al., 2013; Avery et al., 2018; Lucas et al., 2014; Preite et al., 2018; Stark et al., 2018), enhanced cell death was also observed in sorted naive (CD62L<sup>+</sup>CD44<sup>lo</sup>) Pik3cd<sup>E1020K/+</sup> CD8<sup>+</sup> T cells post-stimulation (Figure 1B). To determine whether increased death was cell intrinsic, we evaluated cells from mixed bone marrow (BM) chimeras (outline Figure S1E). Wild-type (WT) hosts receiving BM from either WT or Pik3cd<sup>E1020K/+</sup> donors reproduced the cellular phenotype of intact donor mice (Figure S1F, open symbols). In contrast, in mixed BM chimeras, the presence of Pik3cd<sup>E1020K/+</sup> cells converted WT T cells to an activated phenotype, with even higher percentages of CD44<sup>hi</sup> cells than Pik3cd<sup>E1020K/+</sup> cells (Figure S1F, upper panel, closed symbols) (Bier et al., 2019; Jia et al., 2021). Nonetheless, when T cells from mixed chimeras were stimulated in vitro, more Pik3cd<sup>E1020K/+</sup> T cells died than WT cells in the same cultures (Figure S1F, lower panel, closed symbols). Thus, increased cell death of Pik3cd<sup>E1020K/+</sup> T cells was cell intrinsic and not solely the result of prior activation state.

To evaluate antigen-induced cell death in vivo, WT and Pik3cd<sup>E1020K/+</sup> OT-1 cells were adoptively transferred into congenic hosts that were then infected with influenza X31 expressing the OVA<sub>257–264</sub> peptide (outline Figure 1C). Three days post-infection (p.i.), an elevated number of Pik3cd<sup>E1020K/+</sup> OT-1 cells were found in the lung compared to WT counterparts (Figure 1D, left panel). However, a higher percentage of Pik3cd<sup>E1020K/+</sup> OT-1 cells were Annexin-V<sup>+</sup> (Figure 1D, right panel). Inclusion of CAL-101, a selective p110<sup>δ</sup> inhibitor, prevented increased cell death in cultured Pik3cd<sup>E1020K/+</sup> OT-1 cells (Figure 1E), confirming this phenotype was linked to PI3K<sup>δ</sup> activity. Cell death was primarily apoptotic, as it was associated with increased active caspase-3 and cleaved PARP1 (Figures S1G and S1H) and partially blocked by Z-VAD, a pan-caspase inhibitor (Figure 1E), but not by Necrostatin-1, an inhibitor of necroptosis (Figure S1I). A prominent mechanism of apoptosis in activated T cells is mediated by Fas-FasL signals following TCR restimulation. Upon peptide stimulation, Pik3cd<sup>E1020K/+</sup> OT-1 cells rapidly upregulated Fasl mRNA and surface Fasl to a far greater extent than WT cells (Figures 1F and 1G); similar findings were observed with T cells from patients with APDS (Figure S1J). PI3K<sup>δ</sup> inhibition prevented increased Fasl mRNA and surface Fasl induction (Figures 1F and 1G), and blocking Fas/FasL interaction improved viability (Figure 1H). The data suggest that Fasl-Fas-driven apoptosis is a major driver of increased cell death in activated PI3K<sup>δ</sup> T cells.

**FoxO1 represses Fasl expression in T cells**

FoxOs are major PI3K-regulated TFs that are excluded from the nucleus and inactivated by AKT-mediated phosphorylation. Intriguingly, in neutrophils, FoxO3 represses Fasl expression (Jonsson et al., 2005). TCR-stimulated Pik3cd<sup>E1020K/+</sup> T cells exhibited elevated and sustained pAKT<sup>S473</sup> and pFoxO1/3 (Figure 1I, top panels, and Figure 1J; Angulo et al., 2013; Lucas et al., 2014; Preite et al., 2018; Stark et al., 2018), whereas early TCR-mediated signaling, including pZAP70 and pERK, was minimally affected (Figure 1I, bottom panels). Moreover, inhibition of either p110<sup>δ</sup> or AKT decreased Fasl expression and cell death.
Expression of FoxO1AAA, a mutant that cannot be phosphorylated by AKT and is resistant to inactivation by PI3Kδ (Zhang et al., 2002), reduced FasL expression in both WT and Pik3cdE1020K/+ cells (Figure 1K). Thus, FoxO1 functions as a transcriptional repressor of Fasl in T cells.

**Pik3cdE1020K/+ OT-1 exhibit accelerated and enhanced effector function**

When naive CD8+ T cells are activated, they undergo clonal expansion and differentiation into effectors that express cytotoxic molecules such as granzyme B (GzmB) and FasL and anti-viral cytokines (Hashimoto et al., 2019). Like FasL, Pik3cdE1020K/+ OT-1 cells stimulated *ex vivo* produced excessive interferon-γ (IFN-γ) and tumor necrosis factor α (TNF-α) compared to WT OT-1 cells (Figure 2A). This increase was observed with low or high peptide, a weakly stimulating peptide (T4), and even in CD44lo cells, suggesting a loss of quiescence and/or increased capacity to be rapidly activated even under suboptimal conditions (Figures 2A and 2B).

To evaluate CTL function, OT-1 cells were peptide stimulated, cultured with IL-2 to permit acquisition of cytolytic function, and then exposed to target cells (Figure 2C, outline). Surprisingly, surviving Pik3cdE1020K/+ OT-1 cells displayed robust GzmB expression and killed targets efficiently after only 1 day in IL-2, when WT cells had not fully acquired cytolytic effector function (Figures 2D and 2E). By day 7, WT CTLs had strong cytolytic capacity, but Pik3cdE1020K/+ OT-1 cells still expressed slightly more GzmB and killed targets better (Figures 2F and 2G). Pik3cdE1020K/+ OT-1 effectors also exhibited elevated LFA-1 and adhesion to B cell targets (Figures S2A and S2B) as well as increased CD244, a costimulatory receptor that fine-tunes CTL function and killing of EBV-infected targets (Cannons et al., 2018; Zhao et al., 2012; Figure S2A). Thus, hyperactive PI3Kδ drives accelerated and pronounced cytolytic effector function.

**Activated PI3Kδ drives terminal effector T cell differentiation following viral infection**

To evaluate effector phenotypes *in vivo*, mice were infected with lymphocytic choriomeningitis virus (LCMV) Armstrong. Evaluation at day 4 revealed lower viral loads in Pik3cdE1020K/+ mice compared to WT, consistent with their increased cytolytic capacity (Figures S3A and 2D). By day 8, both genotypes cleared infection from the liver and serum (Matloubian et al., 1994; Figure 3SA; data not shown) and had comparable numbers and frequencies of NP396-specific CD8+ T cells (Figures S3B and S3C) with similar phenotypes, although there were slightly higher percentages of KLRG1+CD127− NP396-specific effector cells in the Pik3cdE1020K/+ mice (Figures S3D and S3E). However, by day 15, percentages and numbers of effector-like antigen-specific cells were clearly elevated in Pik3cdE1020K/+ mice with increased KLRG1+ cells that expressed low CD27 and CD127 (IL-7Rα), a marker of memory precursor cells that is regulated by the FoxO1 target KLF2 (Kerdiles et al., 2009; Kim et al., 2013; Figures 3A, 3B, and S3F–S3I).

TCF1 is a positively regulated FoxO1 target that is essential for efficient TCM generation (Jeannet et al., 2010; Zhou et al., 2010). On day 8, both WT and Pik3cdE1020K/+ antigen-specific T cells showed a similar bifurcation of GzmBhi versus TCF1hi cells. However, WT cells also displayed a small yet discernable TCF1hiGzmBlo population (Figure S3J,
arrow). By day 15, most Pik3cd<sup>E1020K/+</sup> NP396-specific CD8<sup>+</sup> T cells maintained high GzmB expression yet failed to sustain a clear TCF1<sup>hi</sup> population, unlike WT cells (Figure 3C). Moreover, although both WT and Pik3cd<sup>E1020K/+</sup> OT-1 cells initially upregulated TCF1 when activated in vitro, Pik3cd<sup>E1020K/+</sup> CTLs had reduced TCF1 levels after expansion in IL-2 (Figure S3K). Day 15 p.i. Pik3cd<sup>E1020K/+</sup> tetramer<sup>+</sup> CD8<sup>+</sup> T cells also had reduced Eomes, despite equivalent T-bet expression as WT (Figure S3L). Thus, Pik3cd<sup>E1020K/+</sup> CD8<sup>+</sup> T cells display a skewed differentiation toward effector cell phenotypes.

On day 58 post-LCMV infection, similar frequencies of antigen-specific T cells were observed in WT and Pik3cd<sup>E1020K/+</sup> splenocytes, yet Pik3cd<sup>E1020K/+</sup> mice had an increase in the absolute number of tetramer<sup>+</sup> CD8<sup>+</sup> T cells (Figures S3B and S3C), perhaps secondary to increased T cell numbers in lymphoid organs as the mice age (Preite et al., 2018). To evaluate memory cells, we assessed expression of the T<sub>CM</sub> marker, CD62L as well as CD44 (Jeannet et al., 2010; Zhou et al., 2010). Antigen-specific CD8<sup>+</sup> T cells from Pik3cd<sup>E1020K/+</sup> mice displayed an elevated frequency and number of CD44<sup>hi</sup>CD62L<sup>lo</sup> effector-like memory with a reduction in the frequency of CD44<sup>hi</sup>CD62L<sup>hi</sup> T<sub>CM</sub> cells that were CD127<sup>hi</sup> compared to WT mice (Figures 3D, 3E, S3N, and S3O). Thus, many of the CD44<sup>+</sup>CD62L<sup>lo</sup> cells exhibited features of recently described LLECs or terminal T<sub>EM</sub> cells (Milner et al., 2020; Olson et al., 2013; Renkema et al., 2020). Antigen-specific Pik3cd<sup>E1020K/+</sup> cells also expressed less IL-2 (Figure S3P) and high KLRG1 (Figure S3Q), similar to LLECs. Furthermore, NP396-specific Pik3cd<sup>E1020K/+</sup> CD8<sup>+</sup> T cells expressed lower TCF1 than WT cells on day 58 (Figure 3F and S3R). Notably, alloreactive CD8<sup>+</sup> T cells cultured from patients with APDS also failed to maintain a TCF1<sup>hi</sup> population compared to healthy control cells, providing evidence for parallel phenotypes in human cells (Figure 3G). Thus, Pik3cd<sup>E1020K/+</sup> antigen-specific cells expressed decreased CD27 and CD127 (Figures 3E and S3O). Thus, many of the CD44<sup>+</sup>CD62L<sup>lo</sup> cells exhibited features of recently described LLECs or terminal T<sub>EM</sub> cells. Activated PI3Kδ impairs memory T cell expansion following viral infection

To determine whether Pik3cd<sup>E1020K/+</sup> CD8<sup>+</sup> T cells develop functional memory, mice were infected with X31 (H3N2) influenza virus followed by challenge with PR8 (H1N1) influenza on day 30 (Figure 3H). These mouse-adapted strains do not elicit antibody-cross reactivity, allowing for study of CD8<sup>+</sup> T cell memory (Rutigliano et al., 2010; Sabbagh et al., 2006). Pik3cd<sup>E1020K/+</sup> mice cleared X31 more rapidly, with reduced viral titers compared to WT mice on day 4. By day 8, both genotypes had cleared the virus from the lung (Figure S4A), but Pik3cd<sup>E1020K/+</sup> mice showed increased accumulation of PA224-specific CD8<sup>+</sup> T cells compared to WT mice (Figure 3I). Percentages of Pik3cd<sup>E1020K/+</sup> CD8<sup>+</sup> T cells producing effector cytokines IFN-γ and TNF-α in response to PA<sub>224</sub>–233 or anti-CD3 plus anti-CD28 were also elevated (Figure 3J).

NP366-specific cells are a subdominant primary response to X31 but the major population that expands upon PR8 challenge (Rutigliano et al., 2010; Sabbagh et al., 2006). Similar numbers of NP366-specific cells were generated in WT and Pik3cd<sup>E1020K/+</sup> mice upon X31 infection (Figure 3K). However, Pik3cd<sup>E1020K/+</sup> NP366-specific cells expanded poorly.
in response to PR8 challenge, and the magnitude of the secondary NP366-specific response in \( \text{Pik}^3\text{cd}^{E1020K/+} \) mice resembled their primary response (Figure 3K). Similarly, lower percentages of \( \text{Pik}^3\text{cd}^{E1020K/+} \) CD8+ T cells from PR8-challenged mice produced effector cytokines than WT cells when stimulated with NP\_366–\_374 peptide (Figure 3L, left panel). In contrast, CD8+ T cells from PR8-challenged \( \text{Pik}^3\text{cd}^{E1020K/+} \) mice mounted a robust polyclonal response to anti-CD3 and CD28 stimulation, with elevated cytokine-producing cells, providing evidence of aberrant immune activation (Figure 3L, right panel). Furthermore, lung viral titers were comparable between \( \text{Pik}^3\text{cd}^{E1020K/+} \) and WT mice, suggesting that \( \text{Pik}^3\text{cd}^{E1020K/+} \) mice were able to control viral load, consistent with functional LLECs (Figure S4A).

To determine whether aberrant T cell phenotypes in \( \text{Pik}^3\text{cd}^{E1020K/+} \) mice were cell intrinsic and not secondary to altered responses to a subdominant recall antigen or differences in viral milieu, we transferred WT and \( \text{Pik}^3\text{cd}^{E1020K/+} \) OT-1 cells into congenic hosts and infected mice with X31-OVA followed by PR8-OVA challenge (Figure 3M). \( \text{Pik}^3\text{cd}^{E1020K/+} \) OT-1 cells mounted robust primary responses with increased KLRG1hi CD127lo effector cells (Figure S4B) and elevated expression of Blimp-1, a critical TF for effector CD8+ T cell programming that is induced by IL-2 and repressed by BACH2, another TF that is inactivated by AKT and controls T cell quiescence, senescence, and survival (Ando et al., 2016; Figure S4C). However, \( \text{Pik}^3\text{cd}^{E1020K/+} \) OT-1 cells displayed poor secondary expansion, suggestive of impaired memory cell proliferation and/or increased cell death (Figure 3N). Moreover, the TCF1hi population was notably diminished in \( \text{Pik}^3\text{cd}^{E1020K/+} \) OT-1 donor cells, with most cells maintaining high GzmB (Figure 3O), confirming these phenotypes were T cell intrinsic.

### Activated PI3Kδ drives aberrant mTORC1 activity associated with metabolic perturbations

To define molecular and genomic mechanisms by which hyperactive PI3Kδ disturbs CD8+ T cell differentiation, we utilized an *in vitro* culture system where the cellular environment could be finely controlled. Sorted naive (CD62L\_hi CD44\_lo) OT-1 cells were stimulated with antigen for 3 days and evaluated by RNA sequencing (RNA-seq). Transcriptomes for initial naive WT and \( \text{Pik}^3\text{cd}^{E1020K/+} \) OT-1 cells were nearly indistinguishable (Figure 4A, upper panel). In contrast, after 3 days stimulation, 1,200 and 880 annotated transcripts were overrepresented in WT or \( \text{Pik}^3\text{cd}^{E1020K/+} \) cells, respectively (Figure 4A, lower panel). Gene set enrichment analysis (GSEA) of 50 “hallmark” functions and pathways (Subramanian et al., 2005) revealed the mTORC1 pathway was highly enriched in \( \text{Pik}^3\text{cd}^{E1020K/+} \) cells relative to WT (Figures 4B and 4C). mTORC1 is a conserved nutrient sensor that regulates protein synthesis and cell growth, CTL differentiation (Araki et al., 2009; Pollizzi et al., 2015; Sinclair et al., 2008), and induction of the HIF-1α TF, leading to increased glycolytic metabolism that accompanies T cell activation (Finlay et al., 2012; Nizet and Johnson, 2009). Accordingly, \( \text{Pik}^3\text{cd}^{E1020K/+} \) CD8+ T cells displayed enhanced and sustained induction of pS6\_S240/244, a readout of mTORC1 activity (Figure S5A), and increased HIF-1α upon stimulation (Figure 4D).

Next we called differentially expressed genes (DEGs), segregated positively and negatively regulated fractions (mRNA increased or reduced in \( \text{Pik}^3\text{cd}^{E1020K/+} \) relative to WT), and
performed hypergeometric testing (HGT) against gene sets cataloguing computationally defined DNA-binding motifs at gene promoters (Figure 4E; Liberzon et al., 2015). Binding motifs for ARNT (HIF-1β), an obligate partner of HIF-1α, were enriched within positively regulated DEG promoters in activated Pik3cd\textsuperscript{E1020K/+} CD8\textsuperscript{+} T cells. In contrast, DEGs that were higher in WT cells included targets of FoxOs, as well as E12, a component of the E2a TF that is implicated in memory/effector cell decisions (Figure 4E; Masson et al., 2013; Yang et al., 2011).

Consistent with increased pS6 and HIF-1α, antigen-stimulated Pik3cd\textsuperscript{E1020K/+} OT-1 cells displayed elevated GLUT1, a glucose transporter (Figure 4F) and increased lactate, a product of glycolysis, in the media 24 h post-activation (Figure S5B). Evaluation of extracellular acidification rate (ECAR) revealed enhanced glycolysis, glycolytic capacity, and glycolytic reserve in day 3 stimulated Pik3cd\textsuperscript{E1020K/+} OT-1 cells (Figures 4G and S5C), providing evidence that activated PI3Kδ promotes a strong glycolytic profile upon T cell activation.

**PI3Kδ hyperactivity promotes a c-Myc, RNA biogenesis, and aminoacyl-tRNA synthesis program**

In T cells, c-Myc is another major TF that induces expression of genes encoding glycolytic enzymes (Wang et al., 2011). Notably, Myc target genes were the most enriched hallmark set identified by GSEA in Pik3cd\textsuperscript{E1020K/+} transcriptomes, along with multiple Myc-induced pathways, including ribosomal biogenesis, aminoacyl-tRNA synthase, RNA transport, and the unfolded protein response (Figures 4B, 4C, S5D, and S5E). Myc-binding sites were also the most enriched motifs within positively regulated DEG promoters in Pik3cd\textsuperscript{E1020K/+} CD8\textsuperscript{+} T cells (Figure 4E). c-Myc protein was induced similarly in both WT and Pik3cd\textsuperscript{E1020K/+} cells 4 h post-stimulation. However, c-Myc expression diminished progressively in WT cultures but remained high in Pik3cd\textsuperscript{E1020K/+} cells (Figure 4H, left panel). Pik3cd\textsuperscript{E1020K/+} OT-1 cells also exhibited elevated phospho-c-Myc\textsuperscript{S62} post-stimulation (Figure 4H, right panel), suggestive of enhanced c-Myc activity (Preston et al., 2015).

c-Myc induces expression of a broad range of nutrient transporters (Marchingo et al., 2020; Wang et al., 2011); transcripts encoding numerous amino acid (AA) transporters were elevated in Pik3cd\textsuperscript{E1020K/+} cells, including Slc7a5 (leucine, methionine, and tryptophan), Slc1a5 (glutamine, serine, threonine, and alanine), and Slc7a1 (arginine and lysine) (Figure 4I). Surface CD98, a component of the large neutral AA transporter LAT1, and CD71, the transferrin receptor, were also increased (Figure S5F). c-Myc also promotes synthesis of electron transport chain components that augment mitochondrial ATP production to match the high metabolic demands of activated lymphocytes (Singh et al., 2019). Genes involved in oxidative phosphorylation were highly enriched in activated Pik3cd\textsuperscript{E1020K/+} CD8\textsuperscript{+} T cells (Figures 4B and 4C). Accordingly, extracellular flux analyses revealed that activated Pik3cd\textsuperscript{E1020K/+} OT-1 cells had heightened basal oxidative consumption rates (OCRs), increased maximal respiration, and elevated ATP-dependent respiration compared to WT (Figures 4J and S5G). Strikingly, the bioenergetic profile (OCR versus ECAR) showed that activated Pik3cd\textsuperscript{E1020K/+} OT-1 cells were metabolically more energetic than WT.
counterparts (Figures 4K, 4L, and S5H). Thus, hyperactive PI3Kδ leads to a heightened metabolic state reflective of aberrantly activated transcriptional modules downstream of c-Myc and mTORC1.

**Activated Pik3cd<sup>E1020K/+</sup> CD8<sup>+</sup> T cells exhibit pronounced IL-2/STAT5 signatures**

In conjunction with elevated mTOR and c-Myc signatures, GSEA uncovered an enrichment of genes downstream of IL-2/STAT5 in Pik3cd<sup>E1020K/+</sup> CD8<sup>+</sup> T cells (Figures 4B and 5A). These included pro-inflammatory genes, such as Il2ra, Ccr4, Nfil3, and Csf2, and regulatory genes, such as Cish, Socs1, Socs3, Tnfsf10 (encoding TRAIL), and Tnfsf21 (encoding DR6) (Figure 5B). Increased protein was confirmed for several STAT5 targets, including the TF NFIL3 and costimulatory protein TNFRSF9 (CD137), which are both expressed in IL-2 expanded CTLs (Rollings et al., 2018; Figure 5C). Pik3cd<sup>E1020K/+</sup> OT-1 cells also showed elevated BATF3 (Figure 5C), a TF recently implicated in CD8<sup>+</sup> T cell memory (Ataide et al., 2020). NFIL3 and BATF3 were also elevated in CD8<sup>+</sup> T cells from patients with APDS compared to healthy controls following stimulation (Figure 5D).

The IL-2R comprises IL-2Rβ:IL-2Rγ heterodimers and CD25, the high-affinity IL-2Rα chain, which is induced by both TCR and IL-2 signals (Ross and Cantrell, 2018). Stimulation with optimal doses of OVA<sub>257–264</sub> peptide (10 nM) led to similar or only mildly increased CD25 in Pik3cd<sup>E1020K/+</sup> OT-1 cells compared to WT. However, under low peptide dose or altered peptide stimulation, Pik3cd<sup>E1020K/+</sup> OT-1 cells had elevated and sustained CD25 expression (Figure 5E). Stimulated CD8<sup>+</sup> T cells from patients with APDS also exhibited elevated CD25 compared to healthy controls (Figure 5D). Pik3cd<sup>E1020K/+</sup> CD8<sup>+</sup> T cells also produced increased IL-2 (Figure S6A).

IL-2R engagement results in phosphorylation and activation of the STAT5 TF as well as activation of mTORC1 (Ross and Cantrell, 2018). To address whether PI3Kδ hyperactivity also promotes IL-2 responsiveness, OT-1 cells were stimulated under conditions where WT and Pik3cd<sup>E1020K/+</sup> cells expressed similar CD25 (Figure 5E, left panels), washed, and rested. Upon restimulation with IL-2, Pik3cd<sup>E1020K/+</sup> OT-1 cells exhibited elevated and sustained pSTAT5<sup>Y694</sup> and pS6<sup>S240/244</sup> (Figure 5F). To eliminate differential contributions of autocrine IL-2, we included a blocking anti-mouse IL-2 antibody and added saturating levels of exogenous human IL-2 in the cultures prior to resting and re-exposure to IL-2. Under these conditions, Pik3cd<sup>E1020K/+</sup> CD8<sup>+</sup> T cells maintained elevated pS6<sup>S240/244</sup> that increased even further following IL-2 stimulation (Figure 5G). Thus, Pik3cd<sup>E1020K/+</sup> cells exhibit robust and sustained responses to IL-2.

**Activated PI3Kδ drives effector programs at the expense of memory phenotypes**

CD8<sup>+</sup> T cells commit to an effector fate when cultured with IL-2 but differentiate along a memory-like program when cultured with IL-15, a related cytokine that shares two receptor subunits and, at least qualitatively, mobilizes similar signaling pathways (Cornish et al., 2006; van der Windt et al., 2012). To ask whether activated PI3Kδ differentially affects responses to these cytokines, OT-1 cells were peptide-stimulated for 3 days and then cultured with either IL-2 or IL-15 (see Figure S7A).
After expansion in IL-2, WT cultures contained large blasting cells and expressed cytolytic effector molecules and the activation marker CD69 but reduced CD62L. In contrast, WT cells grown in IL-15 were smaller, with low GzmB and elevated CD62L, consistent with a memory-like phenotype (Figures 6A and S7B). Regardless of culture conditions, Pik3cdE1020K/+ OT-1 cells were larger than WT counterparts; Pik3cdE1020K/+ CD8+ T cells cultured in IL-15 had similar diameters as WT cells grown in IL-2 (Figure S7B). In addition, IL-15-cultured Pik3cdE1020K/+ CD8+ T cells exhibited effector-like features, including elevated GzmB and CD69, and low CD62L (Figures 6A and S7B), despite equivalent or higher expression of CD215, the IL-15Rα, compared to WT cells (Figure S7C). Pik3cdE1020K/+ CD8+ cells proliferated more than WT; their proliferation in IL-15 resembled that of WT cells expanded in IL-2 (Figure S7D). Differences in cell death between WT and Pik3cdE1020K/+ CD8+ T cells were also less pronounced after growth in cytokines, particularly in IL-15 (Figure S7E). Pik3cdE1020K/+ CD8+ T cells, therefore, failed to acquire characteristics of TCM cells, even when provided a stimulus that normally pushes toward memory fates.

Principal-component analysis (PCA) of transcripts after in vitro culture with IL-2 or IL-15 confirmed that transcriptomes of Pik3cdE1020K/+ cells exposed to IL-15 broadly resembled those of either WT or Pik3cdE1020K/+ cells exposed to IL-2, as evidenced by increased values on the principal component (PC)2 axis (Figure 6B). This directionality was also seen when comparing WT and Pik3cdE1020K/+ OT-1 cells cultured in IL-2, with the latter exhibiting greater PC2 directionality (Figure 6B).

We then assembled a master set of DEGs across experimental conditions, split it into 10 hierarchical clusters, and ran HGT against Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (Figures 6C and 6D). These clusters revealed distinct patterns that underscored biological differences between WT and Pik3cdE1020K/+ CD8+ T cells. Cluster 4 (red), which contained genes expressed in IL-2-containing cultures, regardless of genotype, was induced by IL-15 only in Pik3cdE1020K/+ cells. This cluster was enriched for genes involved in cytotoxicity, chemokine signaling, and PI3K signaling (Figure 6D). Cluster 9 was enriched for FoxO signature genes; expression of these genes was depressed in Pik3cdE1020K/+ cells regardless of culture conditions (Figures 6C and 6D). In contrast, cluster 2 was enriched for genes involved in ribosomal biogenesis and AA biosynthesis; expression of these genes was exaggerated in Pik3cdE1020K/+ cells cultured in either IL-2 or IL-15, but more so in the former (Figures 6C and S7F), supporting amplification of protein synthesis machinery in Pik3cdE1020K/+ cells.

We next ran HGT against curated sets of effector and memory T cell transcripts derived from mouse models of viral infection (Kaech et al., 2002; Luckey et al., 2006). Cluster 6 (blue), which contained genes highly expressed in WT IL-15 cultures and reduced in Pik3cdE1020K/+ cells, was enriched for genes associated with T cell memory. In contrast, cluster 4, which was expressed by IL-2-treated cultures of both genotypes, as well as Pik3cdE1020K/+ IL-15 cultures, was enriched for genes associated with effector T cells (Figures 6C, 6E, and S7G).
WT effector CD8+ T cells display reduced oxidative metabolism with little to no spare respiratory capacity (SRC) compared to memory cells (Gattinoni et al., 2009; van der Windt et al., 2012). Compared to WT effectors, Pik3cdE1020K/+ OT-1 cells cultured in IL-2 exhibited decreased maximal respiration and SRC (Figures 6F and S7H); these differences were even more profound in IL-15 cultures (Figures 6G and S7I). Thus, Pik3cdE1020K/+ OT-1 cells differentiated in the presence of either IL-2 or IL-15 display impaired cellular fitness with reduced energy reserves compared to WT counterparts.

Memory cells have increased fatty acid oxidation, which is facilitated by expression of lysosomal acid lipase (LAL) (O’Sullivan et al., 2014; WT OT-1 cells cultured in IL-15 expressed more LAL than IL-2-differentiated cells. However, Pik3cdE1020K/+ CD8+ T cells differentiated under either IL-2 effector-like or IL-15 memory-like conditions expressed low LAL (Figure 6H). Collectively, these data provide evidence that IL-15-cultured Pik3cdE1020K/+ OT-1 cells fail to acquire characteristics of memory-like cells and instead have transcriptional and metabolic programs resembling effector cells.

**Activated PI3Kδ alters the chromatin landscape of activated CD8+ T cells**

To provide insight into dysregulated effector/memory programs in Pik3cdE1020K/+ CD8+ T cells, we assessed their chromatin landscape. High-resolution profiling by assay for transposase-accessible chromatin with high-throughput sequencing (ATAC-seq) revealed divergence in areas of open chromatin between activated WT and Pik3cdE1020K/+ CD8+ T cells, with 2,637 unique peaks detected in the former and 1,389 unique peaks in the latter (Figure 7A). Unbiased de novo DNA motif analysis uncovered distinct patterns of TF binding sites within these accessible regions. Consistent with their activated phenotype, JunB-binding sites were the most enriched motif in CD8+ T cells from Pik3cdE1020K/+ mice (Figure 7B; Yukawa et al., 2020). Recognition sites for STAT5 were also among the top enriched motifs in Pik3cdE1020K/+ CD8+ T cells (Figure 7B), with enhanced accessibility at the classic STAT5 targets Socs1, Cish, and Ccr4, as well as Batf3 and Ccl3 (Figures 7C and S8A). Together, these data demonstrate that post-activation, the chromatin landscapes of WT and Pik3cdE1020K/+ CD8+ T cells diverged, with the latter displaying evidence for enhanced STAT5 activity.

Although IL-2 is required for both CD8+ memory and effector T cells, high CD25 expression is linked to effector differentiation (Kalia and Sarkar, 2018; Pipkin et al., 2010). The early difference in STAT5 markings suggested that increased IL-2 production and responsiveness of Pik3cdE1020K/+ CD8+ T cells may contribute to the inability to differentiate along a memory pathway. To address this, we cultured OT-1 cells with blocking anti-IL-2 antibodies or a PI3Kδ inhibitor for 3 days and then washed out the inhibitors and examined differentiation into effector- or memory-like cells upon exposure to IL-2 or IL-15 (Figure S8B, outline). Under these conditions, Pik3cdE1020K/+ OT-1 cells exhibited reduced GzmB and CD25 and re-expressed CD62L by day 3 of activation (Figure 7D, top panel, and Figures S8C and S8D). Nonetheless, when CAL-101 (Figure S8D) or anti-IL-2 (Figure 7D, middle panels) was washed out and cells were exposed to IL-2, Pik3cdE1020K/+ OT-1 cells again expressed higher GzmB and reduced CD62L relative to WT, confirming enhanced IL-2 responses.
In contrast, when the PI3Kδ inhibitor or anti-IL-2 was removed and cells were exposed to IL-15, \textit{Pik3cd}^{E1020K/+} CD8\(^+\) T cells exhibited more memory-like phenotypes, with reduced GzmB and increased CD62L (Figures 7D and S8D, bottom panels). Thus, early exposure of \textit{Pik3cd}^{E1020K/+} CD8\(^+\) T cells to IL-2 results in changes in chromatin and prevents IL-15-induced differentiation into memory-like cells.

**Activated PI3Kδ CD8\(^+\) T cells lose TCF1-associated chromatin accessibility**

Further evaluation of day 3 ATAC-seq data revealed differentially enriched DNA motifs specifically in WT CD8\(^+\) T cells. These included increases in motifs for Brother of the Regulator of Imprinted Sites/CCCTC-binding factor (BORIS/CTCF), which bind to boundaries of chromatin topologically associating domains to define regulatory modules, supporting an early divergence in the overall chromatin organization between activated WT and \textit{Pik3cd}^{E1020K/+} cells (Figure 7B, lower panel). Although we observed differential markings of KLF family motifs (Figure S8E), FoxO-binding sites were only modestly enriched (p = 1e-2). However, recognition sites for TCF1 were highly enriched within accessible regions, specifically in WT CD8\(^+\) T cells (Figure 7B). Parallel findings were observed in antigen-specific cells 1 week post-influenza X31-OVA infection, including differences in motifs for BORIS, IRF1, and TCF1 in WT compared to \textit{Pik3cd}^{E1020K/+} cells (Figures S8F and S8G).

TCF1 is highly expressed in both naive and T\(^{CM}\) cells, and TCF1-binding sites are enriched in accessible regions shared between naive and memory cells after acute viral infection (Scott-Browne et al., 2016). Indeed, a majority of accessible regions found only \textit{in-vitro}-activated WT CD8\(^+\) T cells overlapped with peaks previously identified in naive and memory CD8\(^+\) T cells (Shih et al., 2016), unlike those detected only in \textit{Pik3cd}^{E1020K/+} CD8\(^+\) T cells (Figures 7F and S8H). Thus, hyperactive PI3Kδ instigates a loss of chromatin accessibility at loci controlled by TCF1, a key pro-memory TF. Collectively, our findings position PI3Kδ as a key integrator that drives transcriptional, chromatin, and metabolic changes that promote differentiation of effector cells at the expense of central memory.

**DISCUSSION**

Effector cell differentiation and the development of memory are critical for proper adaptive immunity; understanding these processes is therefore of major importance for improving responses to vaccination and infection. Using a mouse model expressing activated PI3Kδ and T cells from patients with APDS, we provide evidence that PI3Kδ is central to a network of factors driving effector T cell differentiation while preventing acquisition of T\(^{CM}\) phenotypes. Our results have implications both for understanding phenotypes of patients with APDS and the regulation of effector and memory responses.

A large proportion of effector T cells are short-lived and terminally differentiated, providing immediate, acute function, then undergoing apoptosis (Backer et al., 2018). Although PI3K is usually associated with cell survival, T cells from both patients with APDS and \textit{Pik3cd}^{E1020K/+} mice exhibit heightened TCR-induced apoptosis (Angulo et al., 2013; Bier et al., 2019), resulting in part from early and increased expression of \textit{Fasl}, a known target of IL-2-STAT5 (Ross and Cantrell, 2018), AKT, and HIF-1α-pathways (Finlay et al., 2012;
Macintyre et al., 2011). We now show that FoxO1 plays a major role in restraining Fasl expression in CD8+ T cells. Recent data indicate that Fas promotes terminal effector differentiation of naive cells in the presence of activated cells expressing FasL (Klebanoff et al., 2016). Thus, increased Fasl may also potentiate effector differentiation, underscoring positive feedback in this pathway. Furthermore, as Fasl-Fas interactions require a second “competency signal” to induce apoptosis in activated cells (Combadière et al., 1998), our results also implicate PI3Kδ activity in Fas signaling. Nonetheless, Fas-mediated cell death is likely not the only cause of diminished viability, as blocking FasL or AKT did not completely prevent PI3Kδ-driven cell death. Furthermore, Pik3cdE1020K/+ CD8+ T cells also show enhanced proliferation, particularly to cytokines, which may account for their paradoxical increased cell numbers over time.

Our data highlight several major pathways affected by activated PI3Kδ that contribute to heightened effector function. First, despite data arguing that mTORC1 activation and Myc expression are independent of PI3Kδ in CD8+ T cells (Finlay et al., 2009; Spinelli et al., 2021), our findings indicate that activated PI3Kδ is sufficient to drive these processes, perhaps due to increased IL-2 signals. Increased phosphorylation of AKT S473 also points to amplified mTORC2 signals, an idea supported by similar observations in Pten-deficient regulatory CD4+ T cells (Shrestha et al., 2015).

Notably, the most dysregulated pathway in Pik3cdE1020K/+ CD8+ T cells was “Myc targets.” Data suggest that c-Myc is a broad amplifier of transcription (Nie et al., 2012) that itself is increased by mTORC1. Increased Myc likely contributes to the overall heightened activation of Pik3cdE1020K/+ T cells, with a more active, synthetic effector phenotype; increased blastogenesis; and elevated Myc-regulated transcripts associated with AA transport, ribosomal biogenesis, tRNA biosynthesis, and the unfolded protein response (Marchingo et al., 2020). In turn, increased AA transporters may help fuel mTOR activation, which also positively impacts biosynthesis and cell size.

Strikingly, we observed an exaggerated IL-2-STAT5 signature in Pik3cdE1020K/+ CD8+ T cells; this strongly correlates with IL-2 signatures in a recent proteomic analyses of CTLs (Rollings et al., 2018). Although both IL-2 and IL-15 transduce signals via the IL-2R common γ-chain, IL-2 drives increased and prolonged pS6 compared to IL-15 (Cornish et al., 2006). It is therefore of note that Pik3cdE1020K/+ CD8+ T cells displayed heightened and sustained pS6 in response to multiple signals. Our data further suggest that accentuated IL-2 signaling prevents Pik3cdE1020K/+ cells from responding appropriately to IL-15. Nonetheless, early addition of high-dose IL-2 to WT cultures was not sufficient to prevent subsequent “memory-like” differentiation in IL-15 (unpublished observations), suggesting that activated PI3Kδ coalesces multiple signals to promote effector phenotypes.

Although activated Pik3cdE1020K/+ CD8+ T cells show both heightened glycolysis and oxygen consumption, further culture of Pik3cdE1020K/+ cells led to decreased oxidative respiration, with a loss of SRC, which is thought to facilitate rapid expansion and long-term survival in memory cells (van der Windt et al., 2012). Reductions in oxidative phosphorylation were also recently seen in another Pik3cdE1020K/+ mouse strain (Jia et al., 2021), albeit after a different length of activation. Our results suggest that Pik3cdE1020K/+
CD8+ T cells are initially more active bioenergetically, but then may “burn out,” losing their ability to maintain oxidative metabolism for cellular energy, and failing to undergo metabolic reprogramming of memory cells. Why Pik3cd<sup>E1020K/+</sup> mice maintain and even increase a population of antigen-specific cells resembling recently described LL EC is less clear, but is reminiscent of increased T<sub>EM</sub> and EBV-specific cells in patients with APDS and may result from the ability of IL-15 to induce proliferation and effector-like phenotypes in activated PI3Kδ cells. Thus, our data indicate that proper regulation of PI3K activity is critical to balance long-lived effector versus memory cell populations and may be an essential signal for generating LL ECs. It is of note that FoxO1-deficient CD8+ T cells also show decreased T<sub>CM</sub> cells and true CD127<sup>+</sup> T<sub>EM</sub> cells yet increased CD127<sup>lo</sup> LL ECs (Milner et al., 2020). Whether these cells maintain long-term responses during chronic infections is an important question. Indeed, cells with a phenotype similar to LL ECs poorly restrain chronic LCMV clone 13 infection (Nolz and Harty, 2011). Also of note, both our initial description (Lucas et al., 2014), and subsequent work (Cannons et al., 2018; Edwards et al., 2019; Lucas et al., 2014) highlight how T cells from patients with APDS exhibit characteristics of senescence, including increased CD57 and shortened telomeres. We propose these phenotypes are features of Pik3cd<sup>E1020K/+</sup> CD8+ T cell differentiation into “supereffectors” that cannot maintain cellular fitness. Their poor proliferative recall responses combined with a loss of naive T cells over time (Preite et al., 2018), as well as effects on expression of molecules such as 2B4 and other immune cells that affect responses to EBV (Cannons et al., 2018; Edwards et al., 2019; Stark et al., 2018), may ultimately compromise the ability to contain certain chronic infections.

Similar to Pik3cd<sup>E1020K/+</sup> CD8+ T cells, those lacking TCF1 do not develop T<sub>CM</sub> cells after acute infection (Zhou et al., 2010). TCF1-deficient cells are also driven to a terminal-effector-like phenotype (Chen et al., 2019) and fail to maintain long-lived responses in chronic infections (Snell et al., 2018; Utzschneider et al., 2016; Wu et al., 2016). TCF1 is expressed in naive cells but suppressed in a large portion of CD8+ T effector cells in acute infection, while it is maintained in T<sub>CM</sub> cells (Lugli et al., 2020) and CD127<sup>+</sup> T<sub>EM</sub> cells (Milner et al., 2020). In culture, TCF1 expression is suppressed in a portion of activated cells and is a marker of asymmetric cell division, a PI3K-dependent process (Lin et al., 2015). Indeed, we find that activated PI3Kδ plays a critical role in suppressing TCF1, in vitro and in vivo, as well as in in-vitro-activated CD8+ T cells from patients with APDS.

It is therefore of interest that TCF1-associated chromatin modifications are shared by naive and memory cells (Scott-Browne et al., 2016), which retain a more open/poised chromatin conformation compared to effector cells (Gray et al., 2017). In this article, we defined epigenetics as the study of chromatin states that regulate transcriptional activities without alteration of DNA sequences. Our ATAC-seq data argue that activated PI3Kδ cells rapidly lose TCF1-associated markings both in vitro and in vivo. The enrichment of BORIS/CTCF motifs within differentially accessible regions further supports a divergence in the overall chromatin architecture between activated WT and Pik3cd<sup>E1020K/+</sup> CD8+ T cells. Thus, PI3Kδ plays a fundamental role in reorienting the chromatin landscape in a manner that favors effector differentiation and excludes memory specifications.
Multiple interconnected factors have been shown to regulate generation of T\textsubscript{CM} cells, including TFs such as FoxO1, TCF1, KLF2, Blimp1, and BACH2; microRNAs such as miR17–92; chromatin remodeling factors such as Ezh2; and metabolic regulators and signaling molecules such as mTORC1, BCAP, and AKT (Araki et al., 2009; Gray et al., 2017; Hand et al., 2010; Kallies et al., 2009; Kim et al., 2013; Lin et al., 2015; Hess Michelini et al., 2013; Roychoudhuri et al., 2016; Singh et al., 2018; Wu et al., 2012). Remarkably, many of these molecules are regulated by or connected to PI3Kδ. Thus, although we think of TFs as master regulators of differentiation, our data suggest that PI3Kδ is a master regulator integral to a network of interacting factors that drive effector versus memory phenotypes and potentially long-term responses.

APDS is associated with multiple recurrent infections; ~40% of patients exhibit high EBV and/or CMV viral titers (Coulter et al., 2017). Our findings provide insight into some of these phenotypes where CD8\textsuperscript{+} T cells are propelled toward an effector phenotype associated with increased cell death and “metabolic fatigue,” preventing the generation of T\textsubscript{CM} cells during acute infection and perhaps a CD8 stem-like progenitor population during chronic infection. Whether these changes affect long-term responses to infection and whether PI3Kδ inhibitors can alter these phenotypes in APDS are important questions. The implications for adoptive T cell therapies, where use of PI3Kδ inhibitors during cell expansion prior to transfer may maintain progenitor and memory pools and thereby extend longevity of therapy, remain important issues to explore.

STAR★METHODS

RESOURCE AVAILABILITY

Lead contact—Further information and requests for resources and reagents should be directed to the lead contact, Pamela L Schwartzberg (pams@nih.gov).

Materials availability—No new unique reagents were generated in this study. Reagents will be made available upon completion of a Material Transfer Agreement.

Additional resources—The clinical trial studies are registered at ClinicalTrials.gov, Identifier: NCT00128973 and Identifier: NCT00001355.

Data and code availability—RNA-Seq and ATAC-Seq are publicly available. Raw and processed sequencing data for RNA-Seq and ATAC-Seq are available from NCBI Gene Expression Omnibus. Accession number is listed in the Key resources table.

This study does not report original code.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODELS AND SUBJECT DETAILS

Patient samples—Human subjects and guardians in this study signed written consent in accordance with Helsinki principles for enrollment in research protocols approved by
NIAID Institutional review board (clinical trial registration number NCT00001355, protocol 05-I-0213 as well as clinical trial registration number NCT00128973, protocol 05–1-0213). Peripheral blood from patient A.I.1 female age 44 (Takeda et al., 2017), patient F.1 female age 9 and patient H.1 female age 16 was used for Figure 5. Peripheral blood from patient A.1 male age 12, BIII.1 female age 14, C.1 female age 15, D.I.1 male age 40, D.II.2 female age 12, F.II.1 female age 17, G.1 female age 12 was used in Figure 5 (Lucas et al., 2014). Patient samples used for Figure 5 were on Sirolimus treatment. Blood from anonymous healthy donors were obtained from the NIH Clinical center under approved protocols. Peripheral blood mononuclear cells were isolated using lymphocyte separation medium gradient centrifugation.

**Mice and infectious models**—Commercially available mice and reagents are described in the Key resources table. Generation of \( \text{Pi3kcd}^{E1020K/+} \) was previously described (Preite et al., 2018). For adoptive transfer experiments, \( \text{Pi3kcd}^{E1020K/+} \) mice were bred to OT-1 as well as OT-1 BLIMP-YFP mice. We used sex and age matched 8–12 week-old mice (both male and female) for experiments and subsequent comparisons. Mice were maintained and treated under specific pathogen free (SPF) conditions in accordance with the guidelines of the NHGRI (protocol G98–3) and NINDS (protocol 1295–14) Animal Care and Use committees at the NIH (Animal Welfare Assurance #A-4149–01). For adoptive transfers \( 3\times10^6 \) (day 3) or \( 1\times10^4 \) (day 8) OT-1 CD8+ T cells were transferred into CD45.1/CD45.2 recipients. Mice were infected with mouse adapted human influenza virus A/PR/8/34 (PR8), A/X/31 (X31), X31 containing ovalbumin X31-OVA or PR8-OVA. Mice were exposed to aerosolized (Glas-Col) 500 TCID in 10ml of saline. Influenza A/PR8/34 (H1N1) shares the immunodominant nucleoprotein (NP) epitope of the HK-X31 strain but differs in the major neutralizing antibody epitopes of the hemagglutinin (H) and neuraminidase (N) proteins (Sabbagh et al., 2006). Expression of virus in the lungs of infected mice was determined by qPCR (Tarasenko et al., 2017). Mice were injected with \( 2\times10^5 \) PFU of LCMV Armstrong strain (Wu et al., 2016). Viral titers in the liver were determined by plaque assay using Vero cells (Wu et al., 2016).

**METHOD DETAILS**

**Bone marrow chimeras**—8 week old WT CD45.1+ CD45.2+ recipients were sub-lethally irradiated (900 rads) using a Cesium source 18hr before retro-orbital transfer of \( 5\times10^6 \) BM cells from 8 week old donors. For mixed BM chimeras, WT CD45.1+ and \( \text{Pi3kcd}^{E1020K/+} \) CD45.2+ (ratio of 4:1) was transferred. Mice were maintained on acid water for 5–6 weeks and analyzed after 8–12 weeks.

**Cell Culture**—OT-1 (2.5\( \times10^6 \)) splenocytes were stimulated with OVA257–264 or T4 peptide for 3 days in complete media (Kapnick et al., 2017). On day 3, cells were washed, counted and 10 IU/ml of recombinant IL-2 or 10ng/ml IL-15 was added to cultures \((2\times10^5 \text{cells/ml})\) (O’Sullivan et al., 2014). Cells were washed, counted, and fresh cytokines added every 24hr. For IL-2 blocking, anti-mouse IL-2 (20μg/ml) or Ig control (20μg/ml) was added to the cultures. For assays including inhibitors: Idelalisib p110δ inhibitor (CAL-101): 2nM, Z-VAD-FKM caspase inhibitor: (100μM), AKTi: 50–200nM, Necrostatin-1: 0.3mM or DMSO vehicle control was added to cultures. Target and flow-based cytotoxicity assays were
previously described (Kapnick et al., 2017). B cells were stimulated with LPS as previously described (Zhao et al., 2012).

**Flow Cytometry**—Antibodies and reagents are described in Key resources table. For staining, single cell suspension were made from spleen or lymph nodes in RPMI (Penn/Strep, L-Glut, 2ME and 10% FBS). After ACK (ammonium chloride) lysis of RBCs, cells were washed once, counted, and resuspended in FACS buffer (PBS, 2% FBS). Cells were incubated with antibodies for 30–45 min on ice. For Intracellular cytokine staining, cells were permeabilized with BD Cytofix/Cytoperm. Intracellular staining of TFs cells were permeabilized using Foxp3 staining buffer kit. Unconjugated antibodies were detected with secondary antibodies conjugated with 488 or 647. For intracellular staining of cytoplasmic phospho-proteins, cells were fixed with 4% PFA, permeabilized with cold methanol at −20°C as described (Gomez-Rodriguez et al., 2016). For short-term cytokine stimulations, cells were incubated in serum free media for 3hr prior to the cytokine addition. The following reagents were used according to manufacture instructions: PhiPhiLux-G1D2 kit, Annexin-V-APC, Live/Dead Fixable Aqua Dead Cell Stain Kit. MHC class I tetramers specific for LCMV and influenza CD8 epitopes were obtained from the NIAID tetramer facility (Emory University, Atlanta, GA). The following gates were applied before identification of specific cell types: FSC-A/SSC-A, exclusion of doublets (FSC-H/FSC-W and SSC-H/SSC-W) live cells (negative for Aqua). Flow cytometry based conjugate assay was performed as previously described (Cannons et al., 2010). Flow cytometry was performed on a LSRII (BD Bioscience) and data analyzed with FlowJo 9.9 software (Treestar).

**Western Blots**—Stimulations and immunoblot analysis was performed as previously described (Cannons et al., 2004). Briefly, T cells were stimulated with plate bound anti-CD3 (5 μg/ml) +/- anti-CD28 (5 μg/ml) for the indicated times. For short term stimulations, T cells were resuspended in serum free media at 10^8/ml and 100μl of T cells were added to a coated 6 well dish. at 37°C At the indicated times, 100μl of 1% SDS in PBS (plus protease inhibitor minitable (Sigma) and sodium orthovanadate) was added to each well followed by the addition of 900ul 1% TritonX in PBS (containing inhibitors). Lysates were sheared through a 25-gauge needle with a 1 cc syringe 5 times. Lysates were spun at 14 krpm for 15 min at 4°C. For longer time courses, 200–400μl T cells (10^7) were stimulated in media in 2% serum at 37°C in a 24 well dish, prior to removal of media and lysis as above. For assays when cells were cultured in cytokines, 10^7 cells were transferred to an Eppendorf tube and spun down, prior to lysis as above. Proteins (in reducing sample buffer) were separated by SDS-PAGE and transferred to nitrocellulose. Membranes blocked with TBS plus 5% BSA, 0.1% Tween-20 were incubated with primary antibodies overnight at 4°C followed by incubation with HRP-conjugated secondary antibodies for 60 minutes. Signals were detected by chemiluminescence.

**Metabolic Assays**—L-Lactate levels were measured in the cell supernatants using a glycolysis cell-based assay kit (Cayman Chemicals). Oxygen consumption rates (OCR) and extracellular acidification rates (ECAR) were measured with the Seahorse XFe96 Analyzer (Agilent). Briefly, a fixed number of sorted CD8^+ T cells were adhered to XF96 cell culture
microplates immediately before assays using Cell-Tak (Corning). Mito Stress tests were performed in response to 1μM oligomycin, 1.5μM fluoro-carbonyl cyanide phenylhydrazone (FCCP), and 1μM antimycin A plus 0.5μM rotenone in XF media (non-buffered RPMI 1640, 25 mM glucose, 2mM L-glutamine, 1mM sodium pyruvate). Basal respiration: (last rate measurement before oligomycin injection - non-mitochondrial respiration rate), Maximal respiration: (maximal rate after FCCP injection - non-mitochondrial respiration rate), ATP production: (last rate measurement before oligomycin injection – minimal rate measurement after oligomycin injection), Spare respiratory capacity as %: (Maximal respiration / Basal respiration) × 100. Glyco Stress tests were performed in response to 25mM glucose, 1μM oligomycin, and 50mM 2-deoxyglucose (2-DG, Sigma) in XF media (non-buffered RPMI 1640 and 2mM L-glutamine) (van der Windt et al., 2012). Glycolysis: (maximal rate measurement before oligomycin injection – last rate measurement before glucose injection), Glycolytic Capacity: (maximal rate measurement after oligomycin injection – last rate measurement before glucose injection), Glycolytic reserve: (Glycolytic capacity - Glycolysis) (van der Windt et al., 2012). Calculations based on Agilent’s Report Generator Guide.

Retroviral transduction—Migr, Migr-Foxo1 and Migr-Foxo1AAA were kindly provided by Y.S. Choi, J. Choi and S. Crotty. Retroviral stocks were generated as previously described (Cannons et al., 2004). For retroviral transduction, OT-1 CD8+ T cells were activated with OVA257–264 for 24hr. Cells were transduced with viral supernatants plus polybrene (8μg/ml) by centrifuging at 2500 rpm for 90 mins at 30°C. Cells were subsequently cultured in fresh media. Expression of cell surface markers were evaluated 24hr post-transduction.

RNA-sequencing and analysis—Viable, CD62L+CD44lo CD8+ T cells were sorted from spleens of WT OT-I or Pik3cdE1020K/OT-1mice (> 99% purity). These were lysed and stored in Trizol reagent either directly ex vivo or following in vitro stimulation. For the latter, blasting, live, CD8+ T cells were sorted after 72hr of antigen exposure (day 3) or upon subsequent culture with IL-2 or IL-15 for 24 or 48hr. 2–3 biological replicates were sequenced per genotype/condition and equal numbers were collected per replicate (40x10^4 cells). Total RNA was isolated by phenol-chloroform extraction with GlycoBlue as co-precipitant (7–15 μg per sample; Life Technologies) and poly(A)+ mRNA enriched by oligo-dT-based magnetic separation. Single-end read libraries were prepared using the NEBNext Ultra RNA Library Prep Kit and sequenced with a HiSeq 2500 instrument (Illumina, San Diego, CA).

50 bp reads (> 20x10^6 per sample) were aligned onto mouse genome build mm9 with TopHat, assembled with Cufflinks and gene-level counts compiled with htseq-count (Anders et al., 2013). Counts were then normalized, differentially expressed genes (DEG) called, and TPM values calculated using edgeR (Anders et al., 2013). To minimize normalization artifacts, transcripts failing to reach an empirically defined count threshold were purged using HTSFilter (Rau et al., 2013). DEG classification indicates > 2 fold pairwise change and Benjamini-Hochberg (BH) adjusted p value < 0.05 (Figure 4A). An offset value was added to all TPM (TPM+1) and those failing to reach a value > 2 TPM in any genotype/condition were excluded, as were micro-RNAs, sno-RNAs and sca-RNAs. prcomp was
used for PCA with TPM+1 values as input. hclust was used for euclidian clustering with the following TPM+1 sets as inputs: (1) genes defined by Gene Set Enrichment Analysis (GSEA) as core enriched elements within the IL-2-STAT5 signaling pathway (Molecular Signatures database hallmark gene sets) (Figure 5B) (Liberzon et al., 2015), and (2) all DEGs called across pairwise comparisons (Figure 6D).

Clusterprofiler was used to mine the KEGG and Molecular Signatures (MSigDB) gene ontology databases (Yu et al., 2012). For GSEA, input genes were ranked based on a composite metric (Log2FC x −log10 pVal) and tested against MSigDB hallmark (Figures 4B, 4C, 5A, and 5B) (Liberzon et al., 2015). For Hypergeometric Testing, input gene sets were defined by: (1) splitting DEGs into negatively (Pik3cdE1020K/WT) and positively (Pik3cdE1020K>WT) regulated fractions (Figure 4E), or (2) hierarchical clustering of compiled DEGs (Figures 6D and 6E), then tested against KEGG, MSigDB TF targets (C3_TFT) and/or MSigDB immunologic signatures (C7) (Liberzon et al., 2015).

GSEA plots were drawn with enrichplot, heatmaps with heatmap and all other plots with ggplot2 or DataGraph (Visual Data Tools Inc). All input data and test results available in Tables S1 and S2. Raw and processed sequencing data are available from the NCBI Gene Expression Omnibus under accession number GSE155799.

**ATAC-Sequencing and analysis**—ATAC-Seq was performed according to a modified published protocol (Corces et al., 2016). Ten thousand cells, isolated as above, were pelleted and washed with 50 μL PBS. After pelleting the nuclei by centrifuging at 500 x g for 10 min at 4°C, the pellets were resuspended in 50 μL transposase mixture including 25 μL of 2×TD buffer (Tagment DNA buffer, 15027866, Illumina), 2.5 μL of TDE1 (Tagment DNA enzyme, 15027865, Illumina), 0.5 μL of 1% digitonin (G9441, Promega), 22 μL of nuclease-free water. The reaction was incubated at 37°C with shaking at 300 rpm for 30 min. The fragmentalized DNAs were then purified using a QIAGEN MinElute kit (28006) in 10μl elution buffer. Transposed fragments were amplified with 10 or 11 cycles of PCR based on the amplification curve using primers described previously (Buenrostro et al., 2013). Once the libraries were purified using a QIAGEN PCR cleanup kit (28106), they were further sequenced for 50 cycles (paired-end reads) on a HiSeq 2500. The following software were used: Bowtie 0.12.8 (Langmead et al., 2009), Homer v4.10 (Heinz et al., 2010), MACS 1.4.2, Python 3.3.2 (https://www.python.org), R 3.4.0 (https://www.R-project.org), RStudio 1.0.143 (https://www.rstudio.com/), Igv 2.3.42. ATAC-Seq data were processed as previously described with minor modifications (Shih et al., 2016). Raw sequencing data were processed with CASAVA 1.8.2 to generate FastQ files. ATAC-Seq reads from two biological replicates for each sample were mapped to the mouse genome (mm9 assembly) using Bowtie 0.12.8. In all cases, redundant reads were removed using FastUniq (Xu et al., 2012). Regions of open chromatin were identified by MACS (version 1.4.2) (Zhang et al., 2008) using a P value threshold of 1x10^-5. Only one mapped read to each unique region of the genome that was less than 175 base pairs was kept and used in peak calling. Peak intensities (‘tags’ column) were normalized as tags-per-10-million reads (RP10M) in the original library and were plotted in R using Rstudio. Differentially accessible genomic regions were determined by merging MACS called ATAC-Seq peaks from different conditions using the mergePeaks module in HOMER. Enrichment of TF
motifs among genomic regions with differential chromatin accessibility were analyzed by the findMotifsGenome module in HOMER. BigWig tracks were generated by HOMER and visualized by Igv. ATAC-Seq datasets for naive and memory CD8+ T cell were downloaded from previous GEO database accession number GEO: GSE77695 (Shih et al., 2016). Cells used for ATAC-Seq were a portion of the cells isolated for RNA-Seq above.

**Data Analysis**—Flow cytometry was performed using LSRII and sort purification was performed on a BD FACS Aria Fusion. All data were analyzed using FlowJo (9). Extracellular Flux analysis were done with Agilent Seahorse XFe96 Analyzer.

**QUANTIFICATION AND STATISTICAL ANALYSIS.**

Data were analyzed via Prism 6 (GraphPad Software) using non-parametric unpaired Mann-Whitney U test for comparison of two unpaired groups. Graphs show mean ± SEM. *p < 0.05, **p < 0.01 or as indicated in the figures.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Activated PI3Kδ drives accelerated effector T cell function and FasL-driven apoptosis
- Activated PI3Kδ increases IL-2, mTOR, and Myc signatures and alters metabolism
- Activated PI3Kδ is associated with decreased TCF1 and altered chromatin
- After infection, PI3Kδ drives terminal and long-lived effector cells but poor TCM
Figure 1. Activated PI3Kδ T cells show increased cell death, FasL, and pFoxO1
(A) Annexin-V+ T cells from healthy controls and patients with APDS stimulated with anti-CD3 plus anti-CD28 (n = 4).
(B) Annexin-V staining of sorted naive (CD62LhiCD44lo) CD8+ cells stimulated with anti-CD3 plus anti-CD28 (n = 3).
(C and D) OT-1 cells were transferred into CD45 congenic hosts subsequently infected with X31-OVA influenza. (C) Experimental outline. (D) Left: live OT-1 cell numbers. Right: Annexin-V+ cells, (n = 2, 2–3 mice/group).
(E–G) OT-1 cells stimulated with OVA257–264 with or without CAL-101 or Z-VAD (n = 3–5). (E) Cell death. (F) Fasl mRNA. (G) Surface FasL Mean fluorescence intensity (MFI) (left) and a representative histogram at 48 h (right).

(H) Viability of OT-1 cells stimulation with or without blocking FasL, 48 h (n = 4).

(I) Top: T cells stimulated with anti-CD3 plus anti-CD28. Immunoblot for pAKT[S473], AKT, pFoxO1[T24]/FoxO3a[T32], and FoxO1. Bottom: T cells stimulated with anti-CD3. Immunoblot for pZAP70[Y319/Y352], ZAP70, pERK[T202/Y204], and ERK (representative blots, n = 3).

(J) pFoxO1[S256] of OT-1 cells stimulated with OVA257–264 (n = 3, representative histogram, with MFI indicated).

(K) Surface FasL on viable GFP+ OT-1 cells retrovirally transduced with Migr (control), Migr-FoxO1, or Migr-FoxO1[AAA] (n = 3, representative histogram). Graphs show mean ± SEM. *p < 0.05. See Figure S1.
Figure 2. Early and enhanced effector phenotype of Pik3cd<sup>E1020K</sup>/OT-1 CD8<sup>+</sup> T cells

(A and B) IFN-γ and TNF-α production from OT-1 cells stimulated with indicated peptide for 3 h. Shown are CD44<sup>hi</sup> (A) and CD44<sup>lo</sup> (B) cells (n = 3, representative flow plots).

(C–G) OT-1 cells were stimulated with OVA<sub>257–264</sub> for 3 days and then expanded in IL-2.

(E) Outline. (D and F) In vitro cytolysis of LPS-activated B cells pulsed with 1 nM OVA<sub>257–264</sub> by day 4 (D) or day 7 (F) CTLs (n = 3). (E and G) GzmB on day 4 (E) and day 7 (G) (n = 3, representative histogram).

Graphs show mean ± SEM. See Figure S2.
Figure 3. Pik3cd<sup>E1020K<sup>+/−</sup> mice fail to develop a robust T<sub>CM</sub> population

(A–F) Viable CD8<sup>+</sup> splenocytes from mice infected with LCMV Armstrong (n = 2, 4–5/group/time point). (A–C) NP396-specific CD8<sup>+</sup> cells day 15 p.i. (A) CD127 and KLRG1 expression (left: representative staining; right: %KLRG1<sup>+</sup>CD127<sup>+</sup>). (B) Representative histogram of CD27 (top) and CD127 (bottom). (C) GzmB and TCF1 staining. Middle: TCF1 MFI; right: GzmB MFI. (D–F) NP396-specific CD8<sup>+</sup> cells day 58 p.i. (D) CD44 and CD62L staining. Representative flow (left), % CD44<sup>hi</sup>CD62L<sup>lo</sup> cells (right). (E) CD127 histograms: CD44<sup>hi</sup>CD62L<sup>lo</sup> (top) and CD44<sup>hi</sup>CD62L<sup>hi</sup> (bottom). (F) TCF1 MFI.
(G) TCF1 staining of allo-reactive CD8+ cells from healthy controls and patients with APDS (n = 2, representative histogram).

(H–L) Mice were infected with X31 and challenged with PR8 (n = 2, 3–5 mice/genotype/time point). (H) Infection outline. (I) PA224-specific CD8+ cell numbers. (J) IFN-γ and TNF-α from day 8 cells stimulated with either PA224-233 (left) or anti-CD3 plus anti-CD28 (right). (K) NP366-specific CD8+ T cell numbers. (L) IFN-γ and TNF-α from day 35 cells stimulated with either NP366-374 (left) or anti-CD3 plus anti-CD28 (right).

(M–O) OT-1 cells were transferred into congenic hosts, infected with influenza X31-OVA and challenged with PR8-OVA (n = 2, 3 mice/genotype/time point). (M) Outline. (N) Viable OT-1 cell numbers. (O) TCF1 and GzmB expression in OT-1 cells on day 35. Representative experiment (n = 2).

Graphs show mean ± SEM. *p < 0.05; **p < 0.01. See Figures S3 and S4.
Figure 4. Enhanced mTORC1-pathways, sustained c-Myc, and metabolic perturbations in activated Pik3cd$^{E1020K/+}$ OT-I T cells

(A) Volcano plot shows log2 fold change and variance (Benjamini-Hochberg [BH] adjusted p value) for pairwise comparison of detectable transcripts from naive (top) and day 3 antigen-stimulated (bottom) OT-I cells. Negatively (Pik3cd$^{E1020K/+} < $ WT) and positively (Pik3cd$^{E1020K/+} > $ WT) DEGs are highlighted in blue and red, respectively.

(B) Radial plot of GSEA top 10 enriched MSigDB hallmark pathways. Distance from center denotes normalized enrichment score (NES). Color indicates directionality. Blue indicates...
enriched in WT, and red indicates enriched in Pik3cd<sup>E1020K/+</sup>. Element size and saturation are proportional to p and q values, respectively.

(C) GSEA plots of enrichment for selected hallmark pathways.

(D) HIF-1α in T cells (n = 2, representative blot).

(E) HGT for top 10 enriched MSigDB TF target sets among negatively (blue) and positively (red) regulated DEGs. Size and color saturations are proportional to gene count (total shown) and p value, respectively.

(F) OT-1 cells expressing GLUT1 (n = 4).

(G) ECAR on day 3 activated OT-1 cells in response to exogenous glucose, oligomycin, and 2-deoxy-D-glucose (n = 3, representative example).

(H) OT-1 cells were peptide stimulated; shown are total c-Myc (left) and phospho-c-Myc<sup>S62</sup> (right) (n = 3, representative example).

(I) Heatmap of log10 transcripts per million (TPM) +1 values for selected nutrient transporter genes (hierarchically clustered rows).

(J–L) OCR and ECAR measured on day 3 activated OT-1 cells in response to oligomycin, fluoro-carbonyl cyanide phenylhydrazone (FCCP), and antimycin A plus rotenone (AA/R) in the presence of glucose (n = 3, representative example). (J) OCR. (K) Basal ECAR. (L) Ratio of basal OCR/ECAR.

Graphs show mean ± SEM. Graph (K) shows mean ± SEM, p < 0.0001. See Figure S5 and Table S1.
Figure 5. Enhanced IL-2/STAT5 signature and IL-2 sensitivity in activated Pik3cd<sup>E1020K/+</sup> OT-1 T cells

(A) GSEA for IL-2-STAT5 hallmark pathway.

(B) Heatmap of log10 TPM + 1 values for genes (hierarchically clustered rows) defined as core enriched elements in (A). Green boxes, TFs; purple boxes, regulatory genes.

(C) NFIL3, CD137, and BATF3 in peptide-stimulated OT-1 cells (n = 4, representative example).
(D) CD25, BATF3, and NFIL3 in T cells from healthy controls and patients with APDS (on treatment with Sirolimus) stimulated with anti-CD3 plus anti-CD28 (n = 3, representative example).

(E) CD25 on OT-1 cells stimulated with OVA_{257–264} or T4 (n = 4, representative example).

(F) OT-1 cells stimulated with 10 nM OVA_{257–264} for 3 days, re-stimulated with IL-2, and evaluated for pSTAT5^{Y694} and pS6^{S240/244} (n = 3, representative example).

(G) pS6^{S240/244} in OT-1 cells were stimulated with 10 nM OVA_{257–264} with or without vehicle control or anti-IL-2 and exogenous human IL-2 and then washed, rested, and re-stimulated with IL-2 (MFI in histogram; n = 4, representative example).

See Figure S6 and Table S1.
Figure 6. IL-15 differentiated Pik3cd<sup>E1020K</sup>+/OT-1 CD8<sup>+</sup> T cells resemble effector cells
(A–E) OT-1 cells activated with OVA<sub>257–264</sub> for 3 days and then cultured with either IL-2 or IL-15 to generate effector-like or memory-like cells, respectively (see Figure S6A).
(A) GzmB, CD69, and CD62L (n=3, representative flow plots). (B–E) RNA-seq analysis.
(B) Principal-component analysis (PCA) of the two most variant TPM data components (WT cells, circles; Pik3cd<sup>E1020K</sup>+/+, triangles). (C) Heatmap of TPM values and Euclidian clustering for DEGs (rows) and experimental groups (columns). (D and E) Enrichment
across 10 row clusters (C) of KEGG pathways (D) or curated CD8$^+$ T cell gene sets (E) from http://www.gsea-msigdb.org/gsea/index.jsp.

(F and G) OCR under basal conditions and in response to mitochondrial inhibitors oligomycin, FCCP, and antimycin A plus rotenone and calculated as the percent SRC in cells differentiated in IL-2 (F) and IL-15 (G) (representative data, n = 4).

(H) LAL, Tip47, and AKT from IL-2 or IL-15 cultured cells (n = 2). Graphs (F and G) show mean ± SEM, percent SRC p < 0.0001.

See Figure S7 and Table S2.
Figure 7. IL-2 drives early effector trajectory of Pik3cd<sup>E1020K/+</sup> CD8<sup>+</sup> cells
(A–C) ATAC-seq analysis of naive and day 3 activated OT-1 cells (n = 2). (A) Normalized
ATAQ-seq tag density comparing chromatin accessibility (fold change > 2; false discovery
rate [FDR] < 0.05; top: naïve; bottom: day 3). (B) De novo unbiased motif discovery
using HOMER. JunB- and STAT5-enriched motifs noted in Pik3cd<sup>E1020K/+</sup> (top) and Tcf7
enriched motif in WT cells (lower). (C) ATAC-seq genomic tracks across IL-2/STAT5
signature genes. Red boxes highlight differences in chromatin accessibility.
(D) OT-1 cells were stimulated with peptide with or without anti-IL-2 for 3 days, washed,
and cultured in either IL-2 or IL-15 (GzmB and CD62L, representative example; n = 3).
(E) ATAC-seq profiles on Tcf7.

(F) Pie chart of overlapping unique ATAC-seq peaks from WT and Pik3cd^{E1020K/+} day 3 activated OT-I cells versus BM naive CD8^{+} T cells. See Figure S8.
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| anti-human CD3 purified (HIT3a) | BD Biosciences | Cat #555336; RRID: AB_395742 |
| anti-human CD28 (purified (CD28.2) | BD Biosciences | Cat #555725; RRID: AB_396068 |
| anti-human CD62L BV 421 (DREG-56) | Biologend | Cat #302616; RRID: AB_493043 |
| anti-human CD4 PerCP Cy5.5 (A16A1) | Biologend | Cat #357413; RRID: AB_2565666 |
| anti-human CD8 APC eFluor780 (RPA-T8) | Thermo Fisher | Cat #47-0088-42; RRID: AB_1272046 |
| anti-human CD25 Alexa 488 (BC960) | Biologend | Cat #302616; RRID: AB_493043 |
| anti-human Granzyme B BV421 (GB11) | BD Biosciences | Cat #563389; RRID: AB_2738175 |
| anti-human pFoxO1 (S256) | Cell Signaling Technologies | Cat #9461 |
| anti-human FoxO1 (C29H4) | Cell Signaling Technologies | Cat #2880; RRID:AB_2106495 |
| anti-human NFIL3 (D5K80) | Cell Signaling Technologies | Cat #14312; RRID:AB_2798446 |
| anti-human BATF3 | LS Bio | Cat LS-C413396-100 |
| anti-human Myc (D84C12) | Cell Signaling Technologies | Cat #5605; RRID:AB_1903938 |
| anti-human pMyc S62 (EPR17924) | ABCAM | Cat #ab185656 |
| anti-human GLUT1 PE(EPR3915) | ABCAM | Cat #ab209449 |
| anti-human TCF1 (C46C7) | Cell Signaling Technologies | Cat #2206; RRID:AB_2199300 |
| anti-mouse CD3 (2C11) | BioXCell | Cat #BE0001-1; RRID: AB_1107634 |
| anti-mouse CD4 BV605 (RM4-5) | Biologend | Cat #100548; RRID: AB_2563054 |
| anti-mouse CD8 APC eFluor780 (53-6.7) | Thermo Fisher | Cat #47-0081-82; RRID: AB_1272185 |
| anti-mouse CD8 PE (53-6.7) | Thermo Fisher | Cat #12-0081-83; RRID: AB_465530 |
| anti-mouse CD1a FITC (M17/4) | Thermo Fisher | Cat #11-0111-85; RRID: AB_464931 |
| anti-mouse CD25 PE-Cy7 (PC61) | Thermo Fisher | Cat #25-0251-82; RRID: AB_469608 |
| anti-mouse CD27 FITC (LG.3A10) | Thermo Fisher | Cat #11-0271-85; RRID: AB_465002 |
| anti-mouse CD28 purified (37.51) | BioXCell | Cat #BE0015-1; RRID: AB_1107624 |
| anti-mouse CD28 APC (37.51) | Thermo Fisher | Cat #17-0281-82; RRID: AB_469374 |
| anti-mouse CD44 AF700 (IM7) | Thermo Fisher | Cat #56-0441-82; RRID: AB_494011 |
| anti-mouse CD44 FITC (IM7) | Thermo Fisher | Cat #11-0441-82; RRID: AB_465045 |
| anti-mouse CD45.1 BV605 (A20) | Biologend | Cat #110738; RRID: AB_2562565 |
| anti-mouse CD45.2 PerCP(104) | Biologend | Cat #109825; RRID: AB_893351 |
| anti-mouse CD62L PerCP5.5 (MEL-14) | Thermo Fisher | Cat #45-0621-82; RRID: AB_996667 |
| anti-mouse CD62L Alexa Fluor488 (MEL-14) | Biologend | Cat #104420; RRID: AB_493376 |
| anti-mouse CD62L APC (MEL-14) | Thermo Fisher | Cat #17-0621-83; RRID: AB_469411 |
| anti-mouse CD62L eFluor 450 (MEL-14) | Thermo Fisher | Cat #48-0621-82; RRID: AB_1963590 |
| anti-mouse CD69 PE-Cy7 (H1.2F3) | Biologend | Cat #104512; RRID: AB_493564 |
| anti-mouse CD71 PE (C2F2) | BD Biosciences | Cat #552367; RRID: AB_394744 |
| anti-mouse CD98 PE-Cy7 (RL388) | Biologend | Cat #128214; RRID: AB_2750547 |
| anti-mouse CD127 eFluor 450 (A7R34) | Thermo Fisher | Cat #48-1271-82; RRID: AB_2016698 |
| anti-mouse CD137 PE (1AH2) | Biologend | Cat #106106; RRID: AB_2287565 |
| anti-mouse CD178 PE (MFL3) | Biologend | Cat #106606; RRID: AB_313279 |
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| anti-mouse CD215 PerCP-eFluor 710 (DNT15Ra) | Thermo Fisher | Cat #46-7149-82; RRID: AB_11150247 |
| anti-mouse CD244 PE-Cy7 (244F4) | Thermo Fisher | Cat #25-2441-82; RRID: AB_2573432 |
| anti-mouse IFN-γ eFluor 450 (XMG1.2) | Thermo Fisher | Cat #48-7311-82; RRID: AB_1834366 |
| anti-mouse TNF-α PE-Cy7 (MO6-XT22BD) | BD Biosciences | Cat #557644; RRID: AB_396761 |
| anti-mouse IL-2 APC (JES6-5H4) | Thermo Fisher | Cat #17-7021-81; RRID: AB_469489 |
| anti-mouse IL-2 purified (S4B6) | BioXCell | Cat #BE0043-1; RRID: AB_1107705 |
| anti-mouse Eomes PerCP-eFluor710 (Dan11mag) | Thermo Fisher | Cat #46-4875-82; RRID: AB_10597455 |
| anti-mouse KLRG1 PE-Cy7 (2F1) | Thermo Fisher | Cat #25-5893-82; RRID: AB_1518768 |
| anti-mouse TCR Vα2 APC (B20.1) | Thermo Fisher | Cat #17-5812-82; RRID: AB_1659733 |
| anti-mouse TCR Vβ5 PE (MR9-4) | BD Biosciences | Cat #553190; RRID: AB_394698 |
| anti-mouse pSTAT5(Y694) PE (47/Stat5) | BD Biosciences | Cat #612567; RRID: AB_399858 |
| anti-mouse/human pS6(S240/244) Alexa 647(D68F8) | Cell Signaling Technologies | Cat #5044; RRID: AB_10829359 |
| anti-mouse/human pS6(S235/236) Alexa 488 (D57.2.2E) | Cell Signaling Technologies | Cat #4803; RRID: AB_916158 |
| Armenian hamster Ig control | Biologen | Cat #400902 |
| Rat anti-mouse IgG2a control | Thermo Fisher | Cat #04-6200; RRID: AB_2532944 |
| anti-human pFoxO1 (T24) / FoxO3a (T32) | Cell Signaling Technologies | Cat #9464; RRID: AB_329842 |
| anti-human FoxO (C29H4) | Cell Signaling Technologies | Cat #2880; RRID: AB_2106495 |
| anti-human Hif1α (D157W) | Cell Signaling Technologies | Cat #36169; RRID: AB_2799095 |
| anti-human LAL | Thermo Fisher | Cat #PA5-27346; RRID: AB_2544822 |
| anti-human Tip47 | Thermo Fisher | Cat #PA1-46161; RRID: AB_2139115 |
| anti-human pAKT (S473) (D9E) | Cell Signaling Technologies | Cat #4060S; RRID: AB_231504 |
| anti-mouse AKT (C67E7) | Cell Signaling Technologies | Cat #4691S; RRID: AB_915783 |
| anti-human p110δ (D1Q7R) | Cell Signaling Technologies | Cat #34050; RRID: AB_2799043 |
| pZAP-70 antibody (Tyr319)/Syk (Tyr352) (65E4) | Cell Signaling Technologies | Cat #2717T; RRID: AB_2218658 |
| ZAP70 antibody (99F2) | Cell Signaling Technologies | Cat #270S; RRID: AB_2273231 |
| pERK antibody (Thr202/Tyr204) (D13.14.4E) | Cell Signaling Technologies | Cat #4370S; RRID: AB_2315112 |
| ERK antibody (137F5) | Cell Signaling Technologies | Cat #4695S; RRID: AB_390779 |
| anti-rabbit 488 | Thermo Fisher | Cat #A32731; RRID: AB_2633280 |
| anti-rabbit 647 | Thermo Fisher | Cat #A21245; RRID: AB_2535813 |
| anti-rabbit HRP | Thermo Fisher | Cat #31460; RRID: AB_228341 |
| anti-mouse HRP | Thermo Fisher | Cat #G-21040; RRID: AB_2536527 |

**Bacterial and virus strains**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| DH5a competent cells | Thermo Fisher | Cat #18265017 |
| Lymphocytic choriomeningitis virus Armstrong strain | Provided by McGavern Lab, NINDS, NIH | N/A |
| Influenza strain X31 | Provided by McGavern Lab, NINDS, NIH | N/A |
| Influenza strain PR8 | Provided by M. Eichelberger, FDA McGuire Lab, NHGRI, NIH | N/A |
| Influenza strain X31-OVA | Provided by McGuire Lab, NHGRI, NIH | N/A |
| Influenza strain PR8-OVA | Provided by McGuire Lab, NHGRI, NIH | N/A |
| REAGENT or RESOURCE                             | SOURCE                              | IDENTIFIER   |
|------------------------------------------------|-------------------------------------|--------------|
| Biological samples                             |                                     |              |
| Blood Healthy Donor                            | NIH Blood Bank                      | N/A          |
| Peripheral blood from patients with APDS       | Provided by H. Su and G. Uzel, NIAID | N/A          |
| Chemicals, peptides, and recombinant proteins  |                                     |              |
| H-2Db LCMV NP<sub>396-404</sub> (FQPQNGQFI) tetramer | NIH Tetramer Facility              | N/A          |
| H-2Db LCMV GP<sub>33-41</sub> (KAVYNFATM) tetramer | NIH Tetramer Facility              | N/A          |
| H-2Db Influenza NP<sub>366-374</sub> (ASNENMETM) tetramer | NIH Tetramer Facility              | N/A          |
| H-2Db Influenza PA<sub>224-233</sub> (SSLENFRAYV) tetramer | NIH Tetramer Facility              | N/A          |
| OVA<sub>257-264</sub> (SIINFEKL)             | AnaSpec                             | Cat #AS-60193-1|
| OVA T4 (SIITFEKL)                              | AnaSpec                             | Cat #AS-64403 |
| Influenza PA<sub>224-233</sub>               | AnaSpec                             | Cat #AS-61636 |
| Influenza NP<sub>366-374</sub>                | AnaSpec                             | Cat #AS-60624 |
| LCMV NP<sub>396-404</sub> (FQPQNGQFI)         | AnaSpec                             | Cat #AS-61700 |
| recombinant human IL-2                        | NIH AIDS Reagent Program            | Cat #136     |
| recombinant mouse IL-15                       | NCI Repository                      | N/A          |
| LIVE/DEAD™ Fixable Aqua 530                   | Thermo Fisher                       | Cat #L34966  |
| LIVE/DEAD™ Fixable Near-IR                    | Thermo Fisher                       | Cat #L34976  |
| Annexin V APC                                 | Biolegend                           | Cat #640920  |
| Annexin V binding buffer                      | Biolegend                           | Cat #422201  |
| CAL-101                                       | Santa Cruz Biotechnology            | Cat #364453  |
| CAL-101, GS1101                               | Selleckchem                         | Cat #S2226   |
| zVAD FMK                                      | R&D Systems                         | Cat #FK001   |
| Akt inhibitor                                  | Calbiochem                          | Cat #1240-17-1MG |
| Necrostatin-1                                  | Cayman Chemical                     | Cat #11658   |
| Liberase DL                                    | Millipore                           | Cat #5401160001 |
| Oligomycin                                     | Sigma                               | CAS # 1404-19-9 |
| Fluoro-carbonyl cyanide phenylhydrazone (FCCP)| Sigma                               | CAS # 370-86-5 |
| Antimycin A                                    | Sigma                               | CAS # 1397-94-0|
| Rotenone                                       | Sigma                               | CAS # 83-79-4 |
| Lymphocyte Separation Medium                   | MP Bio                              | Cat # 50494X |
| Golgi Stop                                     | BD Biosciences                      | Cat # 554724 |
| CellTrace Violet                               | Thermo Fisher                       | Cat #C34557  |
| cOmplete, Mini Protease Inhibitor Cocktail     | Sigma                               | Cat #11836153001 |
| Sodium orthovanadate                           | Sigma                               | Cat # S6508-10G |
| Polybrene                                      | Sigma                               | Cat #TR-1003-G |
| TRizol™ Reagent                                | Thermo Fisher                       | Cat #15596026 |
| PowerUP™ SYBR™ Green Master Mix                | Thermo Fisher                       | Cat# A25742  |
| SuperScript™ IV First-Strand Synthesis         | Thermo Fisher                       | Cat# 18091050 |
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Critical commercial assays | | |
| Fixation/Permeabilization Solution Kit | BD Biosciences | Cat #554717 |
| Foxp3 / Transcription Factor Staining Buffer Set | Thermo Fisher | Cat #00-5523-00 |
| PhiPhLax-G1D2 kit | OncoImmunin Inc | Cat # A304R1G-5 |
| Naive CD8+ T cell isolation kit | MiltenyiBiotec | Cat #130-096-543 |
| T cell enrichment columns | R&D Systems | Cat #MTCC-525 |
| PureLink Viral RNA/DNA Mini Kit | Thermo Fisher | Cat #12280050 |
| RNeasy Plus Mini Kit | QIAGEN | Cat #74136 |
| L-Lactate Assay kit | CyanChem | Cat #700510 |
| Seahorse XFp Cell Mito Stress Test Kit | Agilent | Cat #103010-100 |
| Seahorse XF Glycolysis Stress Test Kit | Agilent | Cat #103020-100 |
| Deposited data | | |
| Bulk raw and processed RNA-Seq data | GEO | GEO: GSE155799 |
| Bulk raw and processed ATAQ-Seq data | GEO | GEO: GSE155799 |
| Experimental models: Cell lines | | |
| 293T | ATCC | CRL-11268 |
| Experimental models: Organisms/strains | | |
| C57BL/6 | The Jackson Laboratory | Stock# 000664 |
| C57BL/6-Tg(TcraTcrl)1100Mjb/J (OT-1) | The Jackson Laboratory | Stock# 003831 |
| B6.Cg-Tg(Prdm1-EYFP)1Mnz/J (Blimp-YFP) | The Jackson Laboratory | Stock# 008828 |
| B6.SJL-PtprcbPepeb/BoyJ (CD45.1) | The Jackson Laboratory | Stock# 002014 |
| Pi3kcdET302/WT | Preite et al., 2018 | N/A |
| Oligonucleotides | | |
| PR8-NS F 5’ TTC ACC ATT GCC TIC TCT TC 3’ | IDT | N/A |
| PR8-NS R 5’ CCC ATT CTC ATT ACT GCT TC 3’ | IDT | N/A |
| PR8-NP F 5’ CAG CTC ATT ATG ACC AAA TG 3’ | IDT | N/A |
| PR8-NP R 5’ TAC CTG CTT CTC AGT TCA AG 3’ | IDT | N/A |
| Beta-actin F 5’ GGC TGT ATT CCC CTC CAT CG 3’ | IDT | N/A |
| Beta-actin R 5’ CCA GTT GGT AAC AAT GCC ATG T 3’ | IDT | N/A |
| Recombinant DNA | | |
| Migr Foxo1 | Provided by Crotty lab La Jolla Institute for Immunology | N/A |
| Migr Foxo1AAAA | Provided by Crotty lab La Jolla Institute for Immunology | N/A |
| Software and algorithms | | |
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| FlowJo v9.9.6       | BD Bioscience | https://www.flowjo.com |
| Graphpad Prism version 7 | GraphPad | https://www.graphpad.com |
| GSEA software | Subramanian et al., 2005 | https://www.gsea-msigdb.org/gsea/index.jsp |
| Adobe Illustrator 2019 | Adobe | https://www.adobe.com |
| MACS 1.4.2 | Zhang et al., 2008 | http://gensoft.pasteur.fr/docs/macs/1.4.2 |
| FastUniq | Xu et al., 2012 | https://sourceforge.net/projects/fastuniq/files/ |
| Homer v4.10 | Heinz et al., 2010 | http://homer.ucsd.edu/homer/ |
| Python 3.3.2 | Python software foundation | https://www.python.org |
| R 3.4.0 | R development core team | https://www.r-project.org |
| RStudio 1.0.143 | RStudio Team | https://www.rstudio.com |
| IGV 2.3.42 | The Broad Institute | http://software.broadinstitute.org/software/igv/igv2.3 |
| CASAVA 1.8.2 | Illumina | http://bioweb.pasteur.fr/packages/pack@casava@1.8.2/ |
| TopHat 2.1.0 | Anders et al., 2013 | https://ccb.jhu.edu/software/tophat/index.shtml |
| Cufflinks 2.2.1 | Anders et al., 2013 | http://cole-trapnell-lab.github.io/cufflinks/ |
| ClusterProfiler | Yu et al., 2012 | https://guangchuangyu.github.io/software/clusterProfiler/ |
| Partek Genomics Suite 6.6 | Partek | https://www.partek.com/partek-genomics-suite/ |
| Bowtie 0.12.8 | Langmead et al., 2009 | http://bowtie-bio.sourceforge.net/index.shtml |

| Other |
|-------|
| Seahorse Analyzer | Aligent | N/A |
| LSRII Analyzer | BD Biosciences | N/A |
| Fortessa Analyzer | BD Biosciences | N/A |
| Aria Cell Sorter | BD Biosciences | N/A |
| Novex WedgeWell 10%, Tris-Glycine | Thermo Fisher | Cat #XP00100BOX |
| Novex WedgeWell12% Tris glyicine gels | Thermo Fisher | Cat #XP00120BOX |
| Trans-Blot Turbo Mini 0.2 μm Nitrocellulose | BIO-RAD | Cat #1704158 |