Ionic immune suppression within the tumour microenvironment limits T cell effector function

Robert Eil†+, Suman K. Vodnala†+, David Clever1, Christopher A. Klebanoff2, Madhusudhanan Sukumar1, Jenny H. Pan1, Douglas C. Palmer3, Alena Gros1,†, Tori N. Yamamoto1, Shashank J. Patel1, Geoffrey C. Guittard1, Zhiya Yu1, Valentina Carbonaro3, Klaus Okkenhaug3, David S. Schrump1, W. Marston Linehan1, Rahul Roychoudhuri3 & Nicholas P. Restifo1

Tumours progress despite being infiltrated by tumour-specific effector T cells1. Tumours contain areas of cellular necrosis, which are associated with poor survival in a variety of cancers2. Here, we show that necrosis releases intracellular potassium ions into the extracellular fluid of mouse and human tumours, causing profound suppression of T cell effector function. Elevation of the extracellular potassium concentration ([K+]e) impairs T cell receptor (TCR)-driven Akt–mTOR phosphorylation and effector programmes. Potassium-mediated suppression of Akt–mTOR signalling and T cell function is dependent upon the activity of the serine/threonine phosphatase PP2A3,4. Although the suppressive effect mediated by elevated [K+]e is independent of changes in plasma membrane potential (Vm), it requires an increase in intracellular potassium ([K+]i). Accordingly, augmenting potassium efflux in extracellularly specific T cells by overexpressing the potassium channel Kc1.3 lowers [K+]e and improves effector functions in vitro and in vivo and enhances tumour clearance and survival in melanoma-bearing mice. These results uncover an ionometric checkpoint that blocks T cell function in tumours and identify potential new strategies for cancer immunotherapy.

The tumour microenvironment is characterized in part by rapidly dividing cancer cells competing for limited local resources5,6. These factors cause dense areas of cellular apoptosis and necrosis2. Tumour necrosis is frequently associated with a poor prognosis2. Additionally, necrosis alters the extracellular milieu in association with the release of intracellular ions7,8. Although tumour-specific T cells harbour reactivity against tumour antigens9, their function is often suppressed within tumours1. Whether local necrosis or consequent ionic imbalances contribute to T cell dysfunction within tumours is unknown.

Multiple lines of investigation have demonstrated that intact ion transport is required for T cell function. Mutations that affect of store-operated Ca2+ entry result in severe combined immunodeficiency (SCID) in humans10. Moreover, voltage-gated Ca2+ channels are essential for T cell function and survival11,12 and mutations in the Mg2+ channel MAGT1 lead to human T cell immunodeficiency13. Finally, overabundance of Na+ and Cl− promotes T cell pathogenicity and autoimmunity via the kinase SGK-1 (ref. 13). Despite ions playing a key role in T cell function, their extracellular concentrations and functional relevance within tumours are poorly understood.

We hypothesized that tumour cell death leads to a local ionic imbalance within the tumour microenvironment. To isolate native undiluted extracellular fluid within tumours, hereafter termed tumour interstitial fluid (TIF), we used a centrifugation method14 as previously described15,16. This enabled us to compare the concentrations of five principal ions in TIF from mouse B16 melanomas or human tumours with those in serum. [K+]e was higher in TIF than in serum in both mouse and human tumours (Fig. 1a, b and Extended Data Fig. 1a) but not in extracellular fluid isolated from healthy tissues (Extended Data Fig. 1b). We also observed a correlation between TIF [K+]e and the density of dying cells within mouse B16 tumours (Fig. 1c). Additionally, experimental induction of cell death or apoptosis in tumour-derived cell lines increased extracellular potassium concentration ([K+]e) (Fig. 1d and Extended Data Fig. 1c, d). Thus, we conclude that the extracellular space within tumours contains elevated [K+]e, which is associated with local cellular apoptosis and necrosis.

We next investigated whether elevated [K+]e affects T cell function. We found that isomorphic elevations in [K+]e produced dose-dependent suppression of TCR-induced cytokine production (Fig. 1e, f). Elevated [K+]e acted independent of toxicity, with other monovalent and divalent ions or inert osmolytes failing to induce similar suppression (Fig. 1f, g and Extended Data Fig. 1e–h). Elevated [K+]e acutely suppressed T cell activation across a range of signal strengths (Extended Data Fig. 1i), in the presence or absence of co-stimulation (Extended Data Fig. 1j), in a non-redundant fashion with tumour-associated co-inhibitory signals (Fig. 1h, i and Extended Data Fig. 2a, b), in CD4+ Th1 and Th17 effector subtype (Extended Data Fig. 2c, d). However, it had no effect on cellular viability (Extended Data Fig. 2e).

We next isolated endogenous human neoantigen-specific tumour infiltrating lymphocytes (TILs), which have been identified as likely mediators of immunotherapy-induced tumour clearance9,16, and found that interferon-γ (IFN-γ) production by these cells in response to their cognate neoepitope was attenuated by elevated [K+]e (Fig. 1j and Extended Data Fig. 2f, g). Elevated [K+]e also led to suppression of target-specific IFN-γ production by T cells genetically engineered to carry a cancer-germline-antigen-specific TCR (Extended Data Fig. 2h). Thus, our data suggest that elevated [K+]e acutely limits the function of mouse and human T cells.

To understand the basis of this suppression of effector function, we investigated the effect of elevated [K+]e on the molecular events driven by TCR engagement. To this end, we briefly activated fluorescence-activated cell sorting (FACS)-purified mouse CD8+ T cells in the presence or absence of elevated [K+]e and found that elevated [K+]e reduced the expression of transcripts induced by TCR stimulation (Fig. 2a, b). Furthermore, gene-set enrichment analysis indicated that elevated [K+]e suppressed genes induced by TCR signalling, NF-κB activation, escape from anergy, the adaptive immune response and cytokine pathways (Supplementary Information 1). Collectively, these data suggest that intratumoural cell death produces elevated [K+]e concentrations that suppress TCR-driven effector programmes.

As elevated [K+]e acutely suppressed TCR-driven transcriptional events, we investigated whether [K+]e could affect TCR-induced signal transduction pathways. Given the role of [K+]e in regulating plasma membrane potential16,19, we initially hypothesized that K+ suppressed

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1National Cancer Institute, National Institutes of Health (NIH), Bethesda, Maryland 20892, USA. 2Center for Cell Engineering and Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, New York 10065, USA. 3Laboratory of Lymphocyte Signalling and Development, The Babraham Institute, Cambridge CB22 3AT, UK. *Present addresses: Department of Surgery, Oregon Health and Sciences University, Portland, Oregon 97239, SA (R.E.); Vall d’Hebron Institute of Oncology VHIO, Vall d’Hebron University Hospital, c/Natzeret, 115-117, Barcelona 08035, Spain (A.G.). †These authors contributed equally to this work. 

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Figure 1 | Elevated [K+]e within TIF silences the TCR-induced anti-tumour function of mouse and human T cells. a, b, Ratiometric representation of TIF to serum values for the indicated ions from mouse (a) and human (b) tumour tissue. Linear regression and 95% CI best fit line representing the relationship between TIF [K+]e and annexin V+ cells per g of tumour. Significance calculated by Pearson’s correlation coefficient. c, Extracellular concentration of electrolytes following induction of cell death as indicated for mouse (left) and human (right) tumour cell lines. d, Isotonic to RPMI 1640 Elevation of [K+]e 400 mM unless otherwise indicated and quantification (f–i), Human TILs stimulated in the indicated conditions with representative flow cytometry (e), additional [K+]e, equal to 40 mM unless otherwise indicated and quantification (f–i). Human TILs stimulated in the indicated conditions with mutated neo-antigen peptide-pulsed target cells (autologous B cells), additional [K+]e = 50 mM. Centre values and error bars represent mean ± s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001 between selected relevant comparisons, two-way ANOVA. a, c, n = 18 biological replicates; b, n = 5 biological replicates; d, n = 4 culture replicates; e–i, n = 3 culture replicates per condition; j, n = 3 culture replicates; representative of three (e–g) or two (h–j) independent experiments.

TCR activation by inducing cellular membrane depolarization (increased Vm) with subsequent dissipation of the electromotive force driving Ca2+ entry. However, we could not detect any changes in TCR-induced Ca2+ flux in the presence of isotonic elevations in [K+]e (40 mM) (Fig. 2c and Extended Data Fig. 3a). Additionally, elevated [K+]e did not affect the phosphorylation of Zap70, Erk1/2 or PLCγ1, or global tyrosine phosphorylation, following TCR ligation (Fig. 2d and Extended Data Fig. 3b, c). However, elevated [K+]e did reduce TCR-induced phosphorylation of Akt and serine/threonine residues targeted by Akt (Fig. 2e–g and Extended Data Fig. 3d), including mTOR and the ribosomal protein S6 (Fig. 2f, g and Extended Data Fig. 3d). Suppression of Akt–mTOR signalling by elevated [K+]e was noticeable at later time points (Extended Data Fig. 5e), was not replicated by other osmolytes (Extended Data Fig. 4a) and was apparent in conditions of hypotonic hyperkalaemia (Extended Data Fig. 4b). Consistent with a role in limiting Akt–mTOR activity20, elevated [K+]e inhibited TCR-induced nutrient consumption (Extended Data Fig. 4c, d) and CD4+ polarization to effector lineages (Extended Data Fig. 4e, f) and promoted the induction of Foxp3+ CD4+ T cells (Extended Data Fig. 4g). We therefore conclude that elevated [K+]e limits TCR-driven effector function via suppression of the Akt–mTOR pathway.

We next aimed to determine how elevated [K+]e suppresses TCR-induced Akt–mTOR phosphorylation. First, we hypothesized that elevated [K+]e inhibits PI3K activity. However, elevated [K+]e had no effect on TCR-induced phosphatidylinositol-3-phosphate (PtdIns3P) accumulation (Fig. 2h), indicating that K+–mediated suppression of Akt signalling was downstream of PI3K activation. Regulation of Akt activity downstream of PI3K is carried out, in part, by serine/threonine

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**Figure 2** [K+]e inhibits TCR-induced transcripts and function by suppressing Akt–mTOR phosphorylation. a, Pie chart representing proportional subpopulations of all transcripts following 2 h re-stimulation of purified CD8+ T cells with anti-CD3/CD28. b, Volcano plot of TCR-induced genes briefly re-stimulated with anti-CD3/CD28 in the indicated conditions. c, TCR cross-linking induced calcium flux of CD8+ cells as measured by the quotient of Fluor3 and FuraRed fluorescence in the indicated conditions. d, Representative phosphoflow cytometry plots following TCR cross-linking in the indicated conditions. e, Immunoblot analysis of the indicated phospho-residues in CD8+ T cells following TCR cross-linking. f, Quantitative phosphoflow analysis of cells activated as in c and d with representative flow cytometry in g, h. Quantification of the indicated phosphatidylinositol species in CD8+ T cells activated via TCR cross-linking in the indicated conditions. Centre values and error bars represent mean ± s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001 between selected relevant comparisons, two-way ANOVA. c–h, Where noted additional [K+]e = 40 mM. a, c, n = 3 biological replicates; d, f, n = 3 technical replicates per data point; h, n = 3 experimental replicates with pooled analysis displayed; d–g, representative of at least three independent experiments.
phosphorylation of pAkt S473 and pS6S235,S236 10 min after TCR cross-linking in the indicated conditions. a, Selected phosphatase inhibitors from a pharmacologic screen for IFN-γ production by CD8+ T cells in the presence of elevated [K+]o, depicted as the ratio of IFN-γ expression in untreated (ctrl)/elevated [K+]o conditions in the presence of indicated phosphatase inhibitors. b, CD8+ T cell phosphorylation of pAktS473 and pS6S235,S236 10 min after TCR cross-linking in the indicated conditions. c, Compiled analysis of IFN-γ production by retrovirally transduced CD8+ T cells in the presence of elevated [K+]o. Thyl.1 T cells following TCR stimulation in the indicated conditions. d, Pictorial representation of the resultant intracellular changes in Vm and [K+]i in the presence of elevated [K+]o. e, Relative cytoplasmic Vm of CD8+ T cells in the indicated conditions represented as relative fluorescence of the voltage-sensitive fluorescent indicator DISBAC(3). f, Pictorial representation of the resultant intracellular changes in Vm and [K+]i in the presence of gramicidin. g, Relative cytoplasmic Vm of CD8+ T cells in the indicated conditions assayed as in e with representative flow cytometry in h, i. IFN-γ production by CD8+ T cells following TCR-induced stimulation in the indicated conditions. j, Representative flow cytometry representing [K+]i of CD8+ T cells as relative fluorescence of the potassium-sensitive dye Asante-Green 4. k, Relative [K+]i of CD8+ T cells in the indicated conditions assayed as relative fluorescence of Asante-Green 4. l, Representative flow cytometry representing cytokine expression by CD8+ T cells following TCR stimulation in the indicated conditions with compiled quantification in m. Centre values and error bars represent mean ± s.e.m. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001 between selected relevant comparisons, two-tailed Student’s t-tests (a–m). Where noted additional [K+]o = 40 mM. a, c, i, l, m, n = at least three culture replicates per data point; e, g, h, j, k, n = 3 technical replicates per data point; representative of at least two (a, b, c, m) or three or more (e, g, i, h, l, k) independent experiments.

RNA interference against the PP2A subunit Ppp2r2d similarly rescued effector function in the presence of elevated [K+]o (Fig. 3c and Extended Data. Fig. 5c, d). Consistent with the mechanistic involvement of Akt–mTOR hypophosphorylation in the suppression of effector function mediated by elevated [K+]o, we found that T cells expressing a constitutively active form of Akt (Akt1_CA) exhibited resistance to the inhibitory effects of high [K+]o (Fig. 3c and Extended Data Fig. 5e). Thus, we conclude that elevated [K+]o drives hypophosphorylation of the Akt–mTOR pathway in a PP2A-dependent manner.

We next aimed to identify the intracellular changes responsible for decreased Akt–mTOR phosphorylation and cytokine production in the presence of elevated [K+]o. As K+ is the principal determinant of phosphatases. To interrogate cytokine production in the presence of elevated [K+]o, we used a pharmacologic screening approach to determine whether selected compounds, including inhibitors of cellular phosphatases, might restore effector function in the presence of elevated [K+]o (Fig. 3a). Notably, okadaic acid, an inhibitor of the serine/threonine phosphatase PP2A21, significantly restored T cell function in the presence of elevated [K+]o.

Moreover, okadaic acid reversed the hypophosphorylation of Akt and S6 caused by elevated [K+]o (Fig. 3b and Extended Data Fig. 5a) in addition to restoring effector function (Fig. 3c and Extended Data Fig. 5b). Similarly, genetic disruption of PP2A function by overexpression of a dominant-negative isoform (PP2A_DN) or by short-hairpin-mediated
conditions. From the indicated histology following TCR stimulation in the indicated Brefeldin A ( + ), we reasoned that suppression of T cell function by high [K+]i would result in analogous suppression of T cell function. We treated cells with the ionophore gramicidin, which increases [K+]i and T cell suppression in the presence of high [K+]i. Consistent with results from other cell types, we quantified the baseline [K+]i, of T cells as ± 3.3 mM. Additions of 20 or 40 mM [K+]i increased [K+]i to ± 5.5 mM or ± 4.4 mM, respectively (Extended Data Fig. 7c–f), whereas brief exposure to ouabain partially reversed elevations in [K+]i, and T cell suppression in the presence of high [K+]i, (Fig. 3m and Extended Data Fig. 6h–k). Together, these data suggest that elevated [K+]i in the presence of elevated extracellular K+ concentrations may result from a combination of augmented Na+–K+–ATPase function and a relative decrease in K+ flow per channel as the dynamically equilibrated chemical gradient between the intracellular and extracellular space, and the absolute reversal potential for K+, is attenuated.

Collectively, our findings suggested that enhancing T cell K+ efflux might increase T cell anti-tumour function. Previous investigations of T-cell-intrinsic K+ transport, focusing on the voltage-gated potassium channel K1.3 encoded by KCna3 and the calcium-gated potassium channel KCa3.1 (Kcnma4), have described dynamic regulation of K+ transport in association with T cell activation and differentiation state22,23. Brief re-stimulation of mouse CD8+ effector cells in vitro (Fig. 2b) revealed acute upregulation of KCna3 mRNA in addition to dynamic expression of transcripts encoding potassium channels, pumps, and regulatory subunits (Supplementary Information 2). Because KCna3 can be induced by TCR activation and has a role in T cell function24, we hypothesized that enforced expression of KCna3 might increase K+ efflux and thereby increase intratumoural T cell effector function. We found that overexpression of KCna3 in mouse T cells (Fig. 4a, b) resulted in lower T cell [K+]i, (Extended Data Fig. 8a) and impaired resistance to elevated [K+]i-mediated suppression of T cell function (Extended Data Fig. 8b). Overexpression or pharmacologic activation of KCa3.1 produced a similar gain of function and resistance to K+–mediated suppression (Extended Data Fig. 8c, d).

To test whether augmented K+ efflux improved T cell function in vivo, we transferred TCR-transgenic Pmel-1 CD8+ mouse T cells transduced with KCna3, or a control retroviral construct, into B16 tumour-bearing mice. First, we noted that KCna3 overexpression in TILs increased Akt–mTOR activation (Fig. 3c) and IFNγ production 6 h after injection with Brefeldin A (d). Consistently, we found that either elevated [K+]i or inhibition of the Na+–K+–ATPase by ouabain, led to the rescue of T cell function in the presence of elevated [K+]i, (Fig. 3m and Extended Data Fig. 6d–k). Consistent with results from other cell types, we quantified the baseline [K+]i, of T cells as ± 3.3 mM. Additions of 20 or 40 mM [K+]i increased [K+]i to ± 4.5 mM or ± 4.4 mM, respectively (Extended Data Fig. 7c–f), whereas brief exposure to ouabain partially reversed elevations in [K+]i, and T cell suppression in the presence of high [K+]i, (Fig. 3m and Extended Data Fig. 6h–k). Together, these data suggest that elevated [K+]i in the presence of elevated extracellular K+ concentrations may result from a combination of augmented Na+–K+–ATPase function and a relative decrease in K+ flow per channel as the dynamically equilibrated chemical gradient between the intracellular and extracellular space, and the absolute reversal potential for K+, is attenuated.

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To extend our observations, we tested whether human TILs from multiple cancer types were suppressed by elevated [K+]i, or alternative treatments that increase [K+]i, in a PP2A-dependent manner. Consistently, we found that either elevated [K+]i, or inhibition of endogenous potassium channels with Ba2+ increased [K+]i, and suppressed effector function in a manner that also required intact PP2A function in human TILs (Fig. 4e and Extended Data Fig. 9a, b).

To test whether the gain of function observed as a result of KCna3 overexpression resulted from increased ion transport, we generated a non-conducting pore-defKT cell, (Kcnma3_PD; W389F)25. KCna3_PD failed to alter [K+]i, cytokine production in vitro (Fig. 4f, g and Extended Data Fig. 9c) or the effector function of transduced mouse TILs (Extended Data Fig. 9d). Moreover, only intact KCna3 resulted in enhanced tumour clearance and host survival in a mouse tumour model (Fig. 4h, i). Collectively, these results indicate that activating cellular K+ efflux can increase the anti-tumour function of adoptively transferred T cells.

Figure 4 | KCna3 overexpression lowers [K+]i, enhances Akt–mTOR signalling and augments anti-tumour effector function to improve tumour clearance and host survival. a, Pictorial representation of the potassium channel K1.3 (KCna3). b, Representative immunoblot analysis of K1.3 protein abundance in CD8+ Pmel-1 cells following retroviral transduction with Ctrl (Empty-Thy1.1) or KCna3-Thy1.1 constructs. c, d, Thy1.1+ Pmel-1 CD45.1+ CD8+ TIL 6–8 days following transfer into B16 melanoma-bearing mice were analysed for indicated phospho-residues (c) or in vivo IFNγ production 6 h after injection with Brefeldin A (d). e, Relative cytokine expression of human TILs originating from the indicated histology following TCR stimulation in the indicated conditions. f, g, Analysis of [K+]i (f) and representative flow cytometry for the expression of the indicated cytokines (g) of CD8+ Thy1.1+ T cells following transduction with retrovirus expressing Ctrl, KCna3 or KCna3_PD Thy1.1+ constructs. h, i, Rates of tumour growth (h) and host survival (i) represented over time following receipt of Pmel-1 CD8+ T cells transduced as in f, g, two-tailed Student’s t-tests (c–e), two-way ANOVA (f), Wilcoxon rank-sum analysis (h), and log-rank of Kaplan–Meier survival estimates (i). Centre values and error bars represent mean ± s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001 between selected relevant comparisons, additional [K+]i = 50 mM in e. c, n = 5 mice per group; d, n = 7 mice per group; e, each symbol represents the mean of n = 3 culture replicates per patient per data point; f, n = 3 technical replicates per data point; h, i, n = at least ten mice per group, representative of three (h) or two (c–i) independent experiments.

resting Vm (refs 18, 19), we first investigated whether increased Vm in the presence of elevated [K+]i provided the source of T cell suppression (Fig. 3d, e). To this end we tested whether modulation of Vm by other means in the presence of low [K+]i would result in analogous suppression of T cell function. We treated cells with the ionophore gramicidin, which increases Vm by forming pores in the plasma membrane permeable to both Na+ and K+ (Fig. 3f–h). However, as gramicidin, unlike elevated [K+]i, increased IFNγ production (Fig. 3i), we reasoned that suppression of T cell function by high [K+]i is independent of the effect of high [K+]i on Vm and rather depends upon [K+]i. Consistent with this hypothesis, elevated [K+]i, raised [K+]i, while gramicidin depleted [K+]i, and reversed the suppression of T cell function induced by high [K+]i, (Fig. 3j–m and Extended Data Fig. 6a–c). Additionally, other pharmacologic interventions that deplete intracellular K+, such as treatment with the ionophore valinomycin or inhibition of the Na+,K+–ATPase by ouabain, led to the rescue of T cell function in the presence of elevated [K+]i (Fig. 3m and Extended Data Fig. 6d–k). Consistent with results from other cell types, we quantified the baseline [K+]i, of T cells as ± 3.3 mM. Additions of 20 or 40 mM [K+]i increased [K+]i to ± 4.5 mM or ± 4.4 mM, respectively (Extended Data Fig. 7c–f), whereas brief exposure to ouabain partially reversed elevations in [K+]i, and T cell suppression in the presence of high [K+]i (Fig. 3m and Extended Data Fig. 6h–k). Together, these data suggest that elevated [K+]i in the presence of elevated extracellular K+ concentrations may result from a combination of augmented Na+–K+–ATPase function and a relative decrease in K+ flow per channel as the dynamically equilibrated chemical gradient between the intracellular and extracellular space, and the absolute reversal potential for K+, is attenuated.
In this study, we have shown that cell death within tumours is associated with elevated $[K^+]_e$, at a level that leads to increased $[K^+]_i$, within T cells, silencing of TCR-induced Akt–mTOR phosphorylation and decreased T cell effector function. Although intact PP2A function was required for $K^+$-mediated suppression of T cell function, $K^+$ did not directly affect PP2A phosphate activity (Extended Data Fig. 9e, f), suggesting that a functional intermediate is involved. Investigations into the function of PP2A have identified several endogenous small molecules and metabolites that can variably affect PP2A to increase or decrease its contextual function.26,27 Future experiments will aim to determine whether $[K^+]_i$, alters the processing, localization or abundance of metabolites that affect PP2A activity. These findings may also shed light on prior observations that changes in $[K^+]_i$, regulate inflammasome activation in macrophages28 and can control cellular peptide and phospholipid processing29,30.

Finally, we found that elevated $[K^+]_i$, suppresses T cell effector function and that anti-tumour T cells reprogrammed to express the potassium transporter Kcna3 exhibited lower $[K^+]_i$, and enhanced effector functions in vitro and in vivo. These data identify a tumour-induced ionic checkpoint acting upon T cell effector function (Extended Data Fig. 10a–c) and show that manipulating the intracellular ionic concentration of anti-tumour T cells can augment disease clearance, with implications for immune-based therapies for cancer.

**Online Content** Methods, along with any additional Extended Data display items and in Supplementary Information.

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Methods

Study approval. Animal experiments were conducted with the approval of the NCI and NIAMS Animal Use and Care Committees. All NIH cancer patients providing human samples were enrolled in clinical trials approved by the NIH Clinical Center and NCI institutional review boards. Each patient signed an informed consent form and received a patient information form before participation.

Mice and cell lines. Pmel-1 (B6.Cg-Tg [TcrTcb]/8SretJj), Rag2−/−, OT-II (B6.Cg-Tg [TcrTcb]/82CbnfJ), and C57BL/6 mice were obtained from Jackson Laboratory. A line of 6–8-week-old male mice were used as recipient hosts for adoptive transfer unless otherwise indicated. We crossed Pmel-1 mice with Ly5.1 mice (B6.SJL-PtpcPepe/Boul) to obtain Pmel-1 Ly5.1 mice. We crossed OT-II mice with Rag2−/− to obtain OT-II Rag−/− mice. All mice were maintained under specific pathogen-free conditions. B16 (H-2Db), a mouse melanoma, transduced as previously described, to express glycoprotein 100 (gp100) with human residues at positions 25–27; EGS to KVP. The Mel624 human melanoma-derived cell line was a gift of S.A.R. Platinum-E ecotropic packaging cells were obtained from Cell Biologics. HLA-A*0201 NY-ESO-1 A375 cells were obtained from ATCC. Cell lines were maintained in DMEM with 10% FBS, 1% glutamine and 1% penicillin–streptomycin.

Cell line authentication. Platinum-E cells were obtained from Cell Biologics following authentication and validation as being mycoplasma free. A375 cells were obtained from ATCC following authentication and validation as being mycoplasma free. Mel624 was authenticated as previously described. Authenticated B16 was obtained from the National Cancer Institute Tumour Repository and validated as being mycoplasma free. Mel624 was authenticated as previously described. Authenticated B16 was obtained from the National Cancer Institute Tumour Repository and validated as being mycoplasma free. Mel624 was authenticated as previously described. Authenticated B16 was obtained from the National Cancer Institute Tumour Repository and validated as being mycoplasma free. Mel624 was authenticated as previously described. Authenticated B16 was obtained from the National Cancer Institute Tumour Repository and validated as being mycoplasma free. Mel624 was authenticated as previously described.

Statistical analysis. Data were compared using either a two-tailed Student’s t-test corrected for multiple comparisons by a Bonferroni adjustment or repeated measures two-way ANOVA, as indicated. Where necessary, the Shapiro–Wilk test was used to test for normality of the underlying sample distribution. Experimental sample sizes were chosen using power calculations with preliminary experiments or were based on previous experience of variability in similar experiments. Samples that had undergone technical failure during processing were excluded from analyses. The Kolmogorov–Smirnov test was used to evaluate the significance between different distributions. For adoptive transfer experiments, recipient mice were randomized before cell transfer. The products of perpendicular tumour diameters were plotted as the mean ± s.e.m. for each data point, and tumour treatment graphs were compared by using the Wilcoxon rank sum test and analysis of animal survival was assessed using a log-rank test. In all cases, p values of less than 0.05 were considered significant. Statistics were calculated using GraphPad Prism 7 software (GraphPad Software Inc.).

Electrolyte analysis of serum and interstitial fluid. We used a previously reported method to isolate tissue interstitial fluid via a centrifugation method. Briefly, e broc tissue was harvested, placed on triple-layered 10-μm nylon mesh and spun at ∼5g for 5 min to remove surface liquid. Next, samples were centrifuged at 400g a previously validated speed at which intracellular contents are not liberated, for an additional 10 min. Flow-through from this step was retained as interstitial fluid sample. Experimental sample sizes were chosen using power calculations with preliminary experiments or were based on previous experience of variability in similar experiments. Samples that had undergone technical failure during processing were excluded from analyses. The Kolmogorov–Smirnov test was used to evaluate the significance between different distributions. For adoptive transfer experiments, recipient mice were randomized before cell transfer. The products of perpendicular tumour diameters were plotted as the mean ± s.e.m. for each data point, and tumour treatment graphs were compared by using the Wilcoxon rank sum test and analysis of animal survival was assessed using a log-rank test. In all cases, p values of less than 0.05 were considered significant. Statistics were calculated using GraphPad Prism 7 software (GraphPad Software Inc.).

External solution formulations. Unless otherwise indicated, re-activation of cells in elevated [K+]e was performed with an isotonic RPMI formulation with an additional 40 mM of potassium for mouse cells and 50 mM for human cells in comparison to the control condition medium. In principal this medium was produced by obtaining a custom formulation of RPMI 1640 from Gibco that was devoid of NaCl. For control conditions, this medium was reconstituted with NaCl to produce a solution equimolar to standard RPMI. Thus, the final inorganic salt concentrations were, in mM: NaCl 103.4, NaHCO3 23.8, Na2HPO4 5.6, KCl 5.3, MgSO4 0.4 and Ca(NO3)2 0.4. For isotonic medium containing an additional 40 mM of potassium for mouse cells and 50 mM for human cells, the NIH Central Clinical Chemistry Laboratory using auto analyser ion selective electrode quantification (Cobas 6000; US Diagnostics). For isotonic medium containing an additional 40 mM of potassium for mouse cells and 50 mM for human cells, the NIH Central Clinical Chemistry Laboratory using auto analyser ion selective electrode quantification (Cobas 6000; US Diagnostics). For isotonic medium containing an additional 40 mM of potassium for mouse cells and 50 mM for human cells, the NIH Central Clinical Chemistry Laboratory using auto analyser ion selective electrode quantification (Cobas 6000; US Diagnostics).

To produce a solution equimolar to standard RPMI. Thus, the final inorganic salt concentrations were, in mM: NaCl 103.4, NaHCO3 23.8, Na2HPO4 5.6, KCl 5.3, MgSO4 0.4 and Ca(NO3)2 0.4. For isotonic medium containing an additional 40 mM of potassium for mouse cells and 50 mM for human cells, the NIH Central Clinical Chemistry Laboratory using auto analyser ion selective electrode quantification (Cobas 6000; US Diagnostics). For isotonic medium containing an additional 40 mM of potassium for mouse cells and 50 mM for human cells, the NIH Central Clinical Chemistry Laboratory using auto analyser ion selective electrode quantification (Cobas 6000; US Diagnostics). For isotonic medium containing an additional 40 mM of potassium for mouse cells and 50 mM for human cells, the NIH Central Clinical Chemistry Laboratory using auto analyser ion selective electrode quantification (Cobas 6000; US Diagnostics). For isotonic medium containing an additional 40 mM of potassium for mouse cells and 50 mM for human cells, the NIH Central Clinical Chemistry Laboratory using auto analyser ion selective electrode quantification (Cobas 6000; US Diagnostics).
subjected to a rapid expansion protocol (REP) using irradiated PBMC at a ratio of 0.8:1 at 37 °C using an XF24 extracellular analyser (Seahorse Bioscience) for determination of extracellular acidification rate and basal oxygen consumption rate. Transfected cells were cultured in complete medium without IL-2 or MTC for 48 h. In order to ensure complete activation, cells were then further expanded via a REP as described above (days 10–23) and subsequently assayed for target-specific effector function in the indicated conditions.

Adaptive cell transfer (ACT) and tumour immunotherapy. For immunotherapy, C57BL/6 were implanted with subcutaneous B16 melanoma (5 × 10^5 cells). At the time of adaptive cell transfer (ACT), 10 days after tumour implantation, mice (n ≥ 5 for all groups) were sub-lethally irradiated (600 cGy), randomized, and injected intravenously with 5 × 10^5 Pmel-1 cells. Tumours were transduced with control or Kcnma3-expressing retrovirus and received intraperitoneal injections of IL-2 in PBS (6 × 10^7 IU per 0.5 ml) once daily for 3 days starting on the day of cell transfer. Tumours were blindly measured using digital callipers. Tumour size was measured in a blinded fashion approximately every two days after transfer and tumour area was calculated as length × width of the tumour. Mice with tumours greater than 400 mm^2 were killed. The products of the peripheral tumour diameters are presented as mean ± s.e.m. at the indicated times after ACT. For functional analysis of transferred Pmel-1 cells, B16 tumour-bearing mice received Pmel-1 cells as above, and 6–8 days following cell transfer mice were injected with 500 μl of 0.5 mg ml^-1 brefeldin A (Sigma) and 6 h later tumours were harvested and processed for live/dead, surface, fixation, and intracellular staining for direct intravivo IPV capture. For ex vivo restimulation, tumours were harvested and processed as above, red cells were lysed with ACK lysis buffer for 2 min at room temperature, then cell suspensions were subjected to live-cell isolation via ficoll density gradient separation (Cedarlane) and stimulated in medium containing leukocyte activation cocktail with Golgiplug (BD biosciences) for 4 h at a final concentration of 2 μl ml^-1.

Viral infection and kinetic analysis. For assessing the response of CDB+ T cells to acute viral infection, 5 × 10^5 transduced and Thy1.1+ enriched Pmel-1 Ly5.1 CD8+ T cells were transferred into recipient Thy1.2 Ly5.2 C57BL/6 mice. Immediately following transfer, mice were infected with rhgp100 10^7 plaque-forming units (PFU). At the indicated time points following transfer, recipient mouse blood was obtained via sub-mandibular venipuncture and analysis for phenotype and enumeration of the congenically identified transferred cells was carried out.

Identification and purification of mutation-specific TILs. Cancer-specific mutations and TILs targeted against those mutations from patients with metastatic melanoma were identified as previously described. For isolation of T cells to
cross-linking and Ca\textsuperscript{2+} influx. Kinetic analyses were performed with the FlowJo software package (TreeStar).

**shRNA mediated Ppp2r2d knockdown.** A pLKO-Thy1.1 construct targeting Ppp2r2d was provided by P.Z. and K.W.W. and lentiviral particles were generated using their protocol\textsuperscript{4}. Bone marrow (BM) cells were collected from the femurs and tibiae of 6–8-week-old donor mice. After red blood cell lysis, hematopoietic stem and progenitor cells were enriched by autoMACS depletion of lineage-positive cells using the Lineage Cell Depletion Kit (Miltenyi Biotec). Negatively selected cells were cultured in chemically defined serum-free medium X-vivo 10 with gentamicin (Lonza) supplemented with l-glutamine (1 \(\mu\)M), IL-3 (5 ng ml\textsuperscript{−1}), FLT-3L (5 ng ml\textsuperscript{−1}) and IL-7 (5 ng ml\textsuperscript{−1}) (Peprotech). The following day, these lineage-depleted BM cells were transduced by spin-infection at 32°C degrees, 2,000 r.p.m for 90 min in the presence of lentiviral supernatant and 5\(\mu\)g ml\textsuperscript{−1} polybrene (Sigma-Aldrich). Cells were incubated for another 2–4 h before tail vein injection into lethally irradiated (1,000 cGy) recipient mice at 1–2 \texttimes\ 10\textsuperscript{6} cells per mouse in 500 l sterile PBS. CD8\textsuperscript{+} Thy1.1\textsuperscript{−}CD44\textsuperscript{−}CD62L\textsuperscript{−} cells were FACs sorted 6–8 weeks after adoptive transfer, activated, and assayed as above.

**PP2A phosphatase assay.** PP2A activity was evaluated after immunoprecipitation using a malachite green phosphatase assay kit as per the manufacturer’s instructions (EMD Millipore).

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Extended Data Figure 1 | See next page for caption.
Extended Data Figure 1 | Extracellular K⁺ release from apoptotic and necrotic cells inhibits T cell effector function. a, [K⁺] in TIF, RPMI medium and mouse serum. b, Ratio of indicated ions in normal human tissue in comparison to serum measured on the day of tissue collection in cancer patients undergoing resection of nearby cancers originating from the same tissue type. c, Representative flow cytometry plots of B16 melanoma tumour cells following the indicated treatment. d, [K⁺], quantification following the indicated treatment. e, f, Representative flow cytometry plots of anti-CD3/CD28-stimulated CD8⁺ T cells cultured in isotonic or hypertonic RPMI medium in the indicated conditions. g, Quantification of f. h, Quantification of cytokine production by CD8⁺ T cells following stimulation in the indicated conditions; elevated Ca²⁺ and Mg²⁺ are 2 mM, in comparison to 0.4 mM for control conditions. i, Cytokine production by T cells across a titration of anti-CD3 in the indicated conditions. j, Representative flow cytometry plots and quantification following anti-CD3/CD28 titration-based activation of CD8⁺ T cells in the indicated conditions. Centre values and error bars represent mean ± s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001 between selected relevant comparisons, two-tailed Student’s t-tests (a–d, h), two-way ANOVA (g, i, j). a, n = at least three biological replicates; b, n = 5 biological replicates; c, d, n = 4 experimental replicates; e–j, n = 3 culture replicates per condition; c–j, representative of at least two independent experiments.
Extended Data Figure 2 | See next page for caption.
Extended Data Figure 2 | Potassium-induced T cell suppression is functionally non-redundant with CTLA-4 and PD-L1 co-inhibitory signals and is present in TIL neoantigen responses. a, b, IFNγ+ from CD8+ cells in the indicated conditions. c, d, Flow cytometry analysis of cytokine production by CD4+ T cells polarized under Th1 (c) or Th17 (d) conditions and subsequently re-activated via immobilized anti-CD3/CD28 in the indicated experimental conditions. e, Annexin V and propidium iodide (PI) staining following activation of primed CD8+ T cells in the indicated conditions. f, Representative flow cytometry plots and quantification of human neo-antigen-selected TILs from three patients (Pt.) stimulated in the indicated conditions with cognate mutated (mut) neo-antigen peptide-pulsed target cells (autologous B cells).

g, Relevant somatic mutation-induced neoeptopes for three patients A–C in f. h, Representative flow cytometry and quantification of peripheral blood leukocytes from three patients transduced with an HLA*A201-restricted NY-ESO-1 TCR were assayed in the indicated conditions for IFNγ production. Additional [K+]o = 40 mM for a–e, [K+]o = 50 mM for f, h; n = 3 culture replicates per patient per data point, representative of two independent experiments. Centre values and error bars represent mean ± s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001, two-tailed Student’s t-tests (a–h). a–h, n = at least three culture replicates per data point and representative of at least two independent experiments.

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Extended Data Figure 3 Elevated \([K^+]_o\) acts independently of TCR-induced tyrosine phosphorylation and \(Ca^{2+}\) to suppress serine/threonine phosphorylation in the Akt–mTOR axis. 

a, Flow cytometry analysis of TCR-induced \(Ca^{2+}\) influx in the indicated conditions (AUC, area under the curve). 

b, Flow cytometry analysis of TCR-induced phosphorylation of the indicated phospho-residues in primed CD8\(^+\) T cells.

c, Immunoblot analysis of phospho-tyrosine (4G10) residues from primed CD8\(^+\) T cells stimulated as above. For immunoblot source image see Supplementary Fig. 1.

d, Flow cytometry analysis of CD8\(^+\) T cells stimulated via TCR-crosslinking for the indicated phospho-residues and representative histograms at early time points. Filled grey histograms represent unstimulated cells.

e, Flow cytometry analysis of the indicated phospho-proteins in CD8\(^+\) T cells stimulated at later time points following immobilized anti-CD3 and anti-CD28 stimulation in the indicated conditions. Elevated \([K^+]_o\) = 40 mM, isotonic. Centre values and error bars represent mean ± s.e.m.; \(*P < 0.05\); \(*P < 0.01\); \(*P < 0.001\); \(*P < 0.0001\) between selected relevant comparisons. Two-tailed Student’s t-tests (a, b), two-way ANOVA (d, e). a, n = 3 biologic replicates per condition; b, d, e, n = 3 technical replicates per data point. Representative of three (a–d) or two (e) independent experiments.

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Suppression of TCR-induced Akt–mTOR signalling by elevated [K⁺]e limits activation-induced nutrient consumption and T cell effector lineage commitment. a, b, Flow cytometry analysis of the indicated phospho-proteins in CD8⁺ T cells stimulated by anti-CD3 and anti-CD28 cross-linking in the indicated conditions. 

c, 2-NBDG uptake in primed CD8⁺ T cells induced by TCR stimulation in the indicated conditions with representative histograms and quantification. 

d, Seahorse XF Bioflux analysis of CD3/CD28 Dynabead-induced extracellular acidification (ECAR) and oxygen consumption rate (OCR) of CD8⁺ T cells in the indicated conditions. e–g, Flow cytometry analysis of CD4⁺ T cells polarized in the indicated experimental condition concurrently with Th1 (e), Th17 (f), or Treg cytokines (g). Elevated [K⁺]e = 40 mM. Centre values and error bars represent mean ± s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001 between selected relevant comparisons; two-way ANOVA (a, b), two-tailed Student’s t-tests (c–g). n = 3 technical (a, b) or culture (c–g) replicates per data point. a–g, Representative of two independent experiments.
Extended Data Figure 5 | Pharmacologic inhibition and genetic disruption of PP2A function restores T cell effector function in elevated [K+]e. a, Flow cytometry analysis of the indicated phospho-proteins in primed CD8+ T cells stimulated via TCR crosslinking in the indicated conditions. b, c, Flow cytometry analysis of CD8+ T cell IFN-γ production following immobilized anti-CD3/CD28 and induced stimulation in the indicated conditions (b), or among cells expressing a PP2A_DN isoform (c). d, Ppp2r2d expression in the indicated populations followed by flow cytometry analysis of IFN-γ production by the populations. e, Flow cytometry analysis of IFN-γ production by CD8+ T cells expressing an Akt1-CA isoform stimulated in the indicated conditions. Centre values and error bars represent mean ± s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001 between selected relevant comparisons. Two-way ANOVA (a), two-tailed Student’s t-tests (b–e). Where noted in a–e, additional [K+]e = 40 mM. a–e, n = 3 culture replicates per condition and representative of at least two independent experiments.
Extended Data Figure 6 | See next page for caption.
Extended Data Figure 6 | Depletion of intracellular potassium restores T cell cytokine production in the presence of elevated [K\(^+\)].

a, b, [K\(^+\)], of CD8\(^+\) T cells in the indicated conditions assayed via relative fluorescence of Asante-Green 4. c, Flow cytometry analysis of IFN-γ production by primed CD8\(^+\) T cells following immobilized anti-CD3/CD28 based activation in the indicated conditions. d, V\(_{\text{m}}\) of CD8\(^+\) T cells in the indicated conditions assayed with the voltage-sensitive fluorescent indicator DiSBAC\(_4\). e, Relative [K\(^+\)], of CD8\(^+\) T cells in the indicated conditions assayed with Asante-Green 4. f, Pictorial representation of the resultant intracellular changes in V\(_{\text{m}}\) and [K\(^+\)], in the presence of valinomycin. g, Flow cytometry analysis of CD8\(^+\) T cells following immobilized anti-CD3/CD28-based re-activation in the indicated conditions. h, V\(_{\text{m}}\) of CD8\(^+\) T cells in the indicated conditions assayed with DiSBAC\(_4\). i, Relative [K\(^+\)], of CD8\(^+\) T cells in the indicated conditions assayed with Asante-Green 4. j, Pictorial representation of the resultant intracellular changes in V\(_{\text{m}}\) and [K\(^+\)], in the presence of ouabain. k, Flow cytometry analysis of CD8\(^+\) T cells following immobilized anti-CD3/CD28-based re-activation in the indicated conditions. Centre values and error bars represent mean ± s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001 between selected relevant comparisons; two-tailed Student’s t-tests (c, g, k). n = 3 technical (a, b, d, e, h, i) or culture (c, g, k) replicates per data point. a–k, Representative of at least two independent experiments.
Extended Data Figure 7  | Elevated [K$^+$], does not permanently affect T cell [K$^+$]$_i$, $V_m$, or subsequent response to K$^+$-induced suppression of effector function. a. Flow cytometry-based calibration of [K$^+$]$_i$. For all values cells were treated with 50μM gramicidin in titrated doses of [K$^+$]$_e$ to provide a known [K$^+$]$_i$. b, Intra-experimental quantification of [K$^+$]$_i$ in control conditions and elevated [K$^+$]$_e$ based on calibration from a. c, Flow cytometry analysis of the relative $V_m$ of CD8$^+$ T cells of the indicated origin using DiSBAC$_4$(3). d, Cells of the indicated origin assayed in the indicated conditions as in c. e, Flow cytometry analysis of relative [K$^+$]$_i$ of CD8$^+$ T cells of the noted origin washed and assayed in indicated conditions, quantified by relative fluorescence of Asante-Green 4. f, Compiled analysis of relative IFN$^+$ production by CD8$^+$ cells of the indicated origin washed and subjected to TCR stimulation in the indicated conditions. Centre values and error bars represent mean ± s.e.m. NS, not significant, between experimental and control conditions as assessed by two-tailed Student's $t$-test (c) or two-way ANOVA (d–f). For chronic conditioning, additional [K$^+$]$_e$ = 40 mM, n = 3 technical (a–e) or culture (f) replicates per condition. a–f, Representative of two independent experiments.
Extended Data Figure 8 | See next page for caption.
Extended Data Figure 8 | Enforced Kcna3 or Kcnn4 expression in CD8+ T cells augments effector function. a, Flow cytometry analysis of CD8+ T cells retrovirally engineered with Ctrl-Thy1.1 or Kcna3-Thy1.1 encoding constructs assayed for relative [K+], quantified by relative fluorescence of Asante-Green 4. b, Flow cytometry analysis of CD8+ T cells following re-activation in the indicated conditions. c, Flow cytometry analysis of CD8+ T cells retrovirally engineered with Ctrl-Thy1.1, Kcna3-Thy1.1, or Kcnn4-Thy1.1 constructs and re-activation in the indicated conditions. d, Flow cytometry analysis of IFNγ+ in CD8+ cells following re-activation in the indicated conditions. e, Flow cytometry analysis of IFNγ production in Pmel-1 CD8+Thy1.1+ TILs 6–8 days after transfer into tumour-bearing hosts following ex vivo re-activation. f, g, Pmel-1 CD8+ T cells retrovirally engineered with Ctrl-Thy1.1 or Kcna3-Thy1.1 constructs and transferred into C57BL/6 hosts in conjunction with human gp10025-33-encoding vaccinia virus and quantified by cell number (f) or surface phenotype (g) found in the blood of recipients. Centre values and error bars represent mean ± s.e.m.; two-tailed Student’s t-tests (a–d), two-way ANOVA (f, g); NS, not significant; *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. n = 3 technical (a) or culture (b–d) replicates per condition; e, n = 7 mice per group, f, g, n = 5 mice per group. a–g, Representative of two independent experiments.
Extended Data Figure 9 | Elevated [K\textsuperscript{+}]-induced suppression of human CD8\textsuperscript{+} TIL effector function requires intact PP2A activity.

\(a\), Flow cytometry analysis of relative [K\textsuperscript{+}] using Asante-Green 4 on human CD8\textsuperscript{+} TILs in the indicated conditions. \(b\), Representative flow cytometry of the cells in \(a\) following TCR-based activation in the indicated conditions; quantification depicted in Fig. 4e. \(c\), Flow cytometry analysis of CD8\textsuperscript{+} T cells retrovirally engineered with Ctrl-Thy1.1, Kcna3-Thy1.1 or Kcna3_PD-Thy1.1 constructs. \(d\), Flow cytometry analysis of Thy1.1\textsuperscript{+} (transduced) Pmel-1 CD45.1\textsuperscript{+}CD8\textsuperscript{+} TILs re-isolated 6 days after transfer into B16 melanoma-bearing mice and re-stimulated ex-vivo.

\(e, f\), Immunoprecipitated PP2A protein complexes isolated and assayed for relative phosphatase activity in titrated concentrations of okadaic acid (\(e\)) or the indicated conditions (\(f\)). Additional [K\textsuperscript{+}] = 40 mM for mouse cells and 50 mM for human cells unless otherwise indicated. Centre values and error bars represent mean ± s.e.m. NS, not significant between selected relevant comparisons; \(*P < 0.05; **P < 0.01; ***P < 0.001; \(****P < 0.0001\); two-tailed Student's t-tests (\(c, f\)). \(n = 3\) technical (\(a, e, f\)) or culture (\(b, c\)) replicates per condition; \(d, n = 5\) mice per group.

\(a-f\), Representative of two independent experiments.
Intratumoural inhibition of T cell effector function via an ionic checkpoint. 

**a**, Healthy tissue contains limited local cellular decay, maintaining the interstitial $[K^+]_i$ close to serum levels. T cells are robustly activated following TCR stimulation. 

**b**, Tumour-intrinsic phenomena produce a high density of cell death within cancers. Cell death leads to release of intracellular $K^+$ into the extracellular space. The resultant elevated $[K^+]_e$ acts to increase the $[K^+]_i$ of T cells, limiting their activation and effector function.

**c**, Reduction of $[K^+]_i$ and increased effector function can be imparted to tumour-specific T cells by overexpression of Kv1.3 ($Kcna3$).

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