Neoantigens derived from somatic mutations are specific to cancer cells and are ideal targets for cancer immunotherapy. KRAS is the most frequently mutated oncogene and drives the pathogenesis of several cancers. Here we show the identification and development of an affinity-enhanced T cell receptor (TCR) that recognizes a peptide derived from the most common KRAS mutant, KRAS<sup>G12D</sup>, presented in the context of HLA-A*11:01. The affinity of the engineered TCR is increased by over one million-fold yet fully able to distinguish KRAS<sup>G12D</sup> over KRAS<sup>WT</sup>. While crystal structures reveal few discernible differences in TCR interactions with KRAS<sup>WT</sup> versus KRAS<sup>G12D</sup>, thermodynamic analysis and molecular dynamics simulations reveal that TCR specificity is driven by differences in indirect electrostatic interactions. The affinity enhanced TCR, fused to a humanized anti-CD3 scFv, enables selective killing of cancer cells expressing KRAS<sup>G12D</sup>. Our work thus reveals a molecular mechanism that drives TCR selectivity and describes a soluble bispecific molecule with therapeutic potential against cancers harboring a common shared neoantigen.
As an intracellular protein, KRAS is inaccessible to conventional monoclonal antibodies, while the lack of an amenable mutant-specific binding pocket has hindered attempts to develop selective small molecule antagonists. Recently, AMG510 (sotorasib) and MRTX849 (adagrasib), inhibitors capable of binding irreversibly to KRAS<sup>G12C</sup>, have demonstrated promising clinical anti-tumor activity. However, these molecules target a less frequent mutation and so novel approaches are required to treat cancers driven by more frequent mutations, such as KRAS<sup>G12D</sup>.

Unlike antibodies that, in the context of cancer recognition, primarily bind to intact cell surface expressed proteins, T cells can access mutated or dysregulated intracellular proteins via T cell receptor (TCR) recognition of neoantigen derived peptides presented by human leukocyte antigen (HLA) class I on the cancer cell surface. Thus, exploiting T cell mediated tumor immunity offers an alternative approach to target cancer specific neoantigens. In support of this approach, a T cell clone specific for a KRAS<sup>G12D</sup> peptide presented in the context of HLA-C*08:02 has been successfully utilized in immunotherapy to suppress tumors in a patient. In addition, a murine TCR isolated from humanized mice, and a bispecific T cell engaging TCR-mimetic antibody targeting KRAS<sup>G12D</sup> peptides in the context of HLA-A*0101 (HLA-A*011) and HLA-A*0301, respectively, have demonstrated T cell activation and killing of cancer cells preclinically. These pioneering studies have opened avenues to target KRAS mutant neoepitopes using peptide-HLA (pHLA) specific therapeutics. However, the mechanisms underlying specificity to these neoantigens remains poorly understood.

Here, we isolate and affinity-enhance a human TCR specific to a KRAS<sup>G12D</sup> decamer peptide (VVVGADGVGK) presented in the context of HLA-A*011 (HLA-A*011 KRAS<sup>G12D</sup>). A combination of structural, biochemical, and computational approaches show that the TCR intimately achieves peptide selectivity from differences in electrostatic interactions, despite minimal changes in direct TCR-pHLA contacts, providing a detailed mechanistic understanding of the selectivity of this TCR to HLA-A*011 KRAS<sup>G12D</sup> over HLA-A*011 KRAS<sup>WT</sup>. Using this affinity enhanced TCR as the targeting arm, a bispecific T cell engaging ImmTAC (Immune mobilizing monoclonal TCR Against Cancer) molecule, mediates selective T cell targeting of HLA-A*011+ cancer cells naturally expressing KRAS<sup>G12D</sup>. This work highlights the exquisite sensitivity of the TCR:pHLA system and implies that soluble high affinity TCR bispecifics may have the potential to treat neoantigen driven cancers.

**Results**

**Identification and characterisation of a KRAS<sup>G12D</sup> specific TCR**

We identified a KRAS<sup>G12D</sup> specific human TCR (JDI TCR) from the peripheral blood mononuclear cells (PBMC) of an HLA-A*011 healthy donor (JDI). T cells transduced with the JDI TCR, encoded by TRAV19<sup>01</sup>01 and TRBV6<sup>2</sup>*01, were co-cultured with peptide-pulsed HLA-A*011+ acute lymphoblastic leukemia SUP-B15 B cells. Concentration dependent IFNγ release was detected in co-cultures of JDI TCR-transduced cells and SUP-B15 cells pulsed with KRAS<sup>G12D</sup> decamer peptide (VVVGADGVGK), but not in response to KRAS<sup>WT</sup> peptide (VVVGAGGVGK), or unpulsed controls (Fig. 1a). Consistent with these data, the JDI TCR bound to HLA-A*011 KRAS<sup>G12D</sup> with an affinity (K<sub>d</sub>) of 63 µM, with no measurable binding affinity to HLA-A*011 KRAS<sup>WT</sup> (Table 1).

To further assess JDI TCR specificity, we analyzed binding to several peptides from other RAS superfamily GTPase proteins with high amino acid sequence similarity, as well as pools of HLA-A*011 presented nonamer/decamer self-peptides from ubiquitously expressed genes (Supplementary Table S1). No measurable binding was detected to any of these pHLAs indicating a high level of selectivity towards KRAS<sup>G12D</sup> (Table 1). Alanine substitution at each position of the peptide indicated that peptide residue D6, along with G4, A5, and G9, were most critical for JDI TCR binding (Fig. 1b), demonstrating the importance of the KRAS<sup>G12D</sup> mutation in mediating TCR recognition.

**Affinity-enhanced JDI TCRs retain the ability to distinguish KRAS<sup>G12D</sup> from KRAS<sup>WT</sup>**

Strengthening TCR affinity enables effective targeting of antigens presented at low levels on the cell surface while longer t<sub>1/2</sub> may improve residence time of the TCR on antigen presenting cells to elicit persistent and effective T cell activation and target cell killing. We affinity enhanced the HLA-A*011 KRAS<sup>G12D</sup> specific JDI TCR using NNK randomisation of complementarity determining regions (CDR) to ensure that discrimination between the neoepitope KRAS<sup>G12D</sup> and KRAS<sup>WT</sup> self-peptide was maintained or enhanced, affinity variant phage libraries were depleted for HLA-A*011 KRAS<sup>WT</sup> binders prior to positive selection on HLA-A*011 KRAS<sup>G12D</sup>. Binding to the ubiquitously expressed HLA-A*011 KRAS<sup>WT</sup> was monitored throughout the affinity maturation process and only the most specific mutants were used to combine and enhance the affinity in an iterative process. This procedure enabled incremental improvements in the JDI TCR affinity for HLA-A*011 KRAS<sup>G12D</sup> (Fig. 1c), whilst widening the affinity window (K<sub>d</sub> KRAS<sup>G12D</sup>/K<sub>d</sub> KRAS<sup>WT</sup> pHLA, Fig. 1d) from 98-fold for the JDIa9bwt TCR to >4000-fold for the affinity enhanced JDI41b1 TCR and JDIa9b535 TCR compared to binding to HLA-A*011 KRAS<sup>WT</sup> (Fig. 1e, Table 2, Supplementary Fig. S1). Several affinity-enhanced JDI TCRs were generated with K<sub>d</sub> in the range of 50-100 pM and t<sub>1/2</sub> ≥ 20 hr representing a one-million-fold affinity enhancement over the parent JDI TCR with enhanced selectivity relative to the ubiquitously expressed HLA-A*011 KRAS<sup>WT</sup>.

The JDIa41b1 TCR adopts a virtually identical binding mode in complex with HLA-A*011 KRAS<sup>G12D</sup> and HLA-A*011 KRAS<sup>WT</sup>

To understand the molecular basis of TCR selectivity for HLA-A*011 KRAS<sup>G12D</sup> we solved the crystal structures of the JDIa41b1 TCR in complex with HLA-A*011 KRAS<sup>G12D</sup> (K<sub>d</sub> = 743 ± 18 pM) and HLA-A*011 KRAS<sup>WT</sup> (K<sub>d</sub> = 3.0 µM) at 2.38 Å (JDIa41b1-HLA-A*011 KRAS<sup>G12D</sup> complex, PDB 7OW6) and 2.64 Å (JDIa41b1-HLA-A*011 KRAS<sup>WT</sup> complex, PDB 7OW5) resolution, respectively (Table 3). A superposition of the JDIa41b1-HLA-A*011 KRAS<sup>G12D</sup> and JDIa41b1-HLA-A*011 KRAS<sup>WT</sup> complexes revealed a virtually identical conformation (Root mean square deviation (RMSD) of TCR variable domains: 0.29 Å for Cα atoms and 0.80 Å for all atoms) (Fig. 2a), an identical crossing angle of 50° (Fig. 2b), with all CDRs, except CDRβ, directly contributing to the binding interface. Composite omit maps of the KRAS<sup>G12D</sup> and KRAS<sup>WT</sup> peptides and TCR CDR regions in the JDIa41b1-HLA-A*011 KRAS<sup>G12D</sup> and JDIa41b1-HLA-A*011 KRAS<sup>WT</sup> complexes, showed that modeled residues at the binding interface agree well with the observed experimental data. For a few CDR residues (R28a, Q57α), the side-chain densities were relatively weak (Supplementary Fig. S2), but the modeled side-chain structures do not differ substantially between the JDIa41b1-HLA-A*011 KRAS<sup>G12D</sup> and JDIa41b1-HLA-A*011 KRAS<sup>WT</sup> complexes. Only R28α contributes significantly to differences in binding affinity (based on the decomposition of binding energy calculated from MD simulations) and is discussed below. Analysis of the JDIa41b1-HLA-A*011 KRAS<sup>G12D</sup> and JDIa41b1-HLA-A*011 KRAS<sup>WT</sup> binding interfaces revealed that both complexes were very similar, with the JDIa41b1 TCR engaging mainly the C-terminus of the peptide through the CDRβ3 (Fig. 2c). Polar contacts to the peptide backbone were made through TCR residues Y33α (to peptide A5), N10β and H100β (to peptide G7) and G98β (to peptide G9).

TCR-HLA interactions were also identical in both complexes and were dominated by TCR α-chain contacts to the HLAα1 helix. Key JDIa41b1 TCR residues at the HLA interface included CDRα residues R28α and D29α that formed salt bridges to HLAα1 residues E85 and R65, respectively, and CDR3a residues P97α, G99α, D100α.

Nature Communications | (2022) 13:5333 
https://doi.org/10.1038/s41467-022-32811-1
and G101α that contacted HLAα1 residues R65, K68 and A69 (Supplementary Fig. S3b). Additional interactions were made through the TCR CDR3β residues P97β, G98β and H100β, that engaged HLAα1 helix residues A69, Q72 and T73, TCR CDR1β residue E30β that formed a salt bridge with HLA residue R75 (Supplementary Fig. S3a), TCR CDR1α residue T30α that bound to HLAα2 residues R163, and TCR CDR2α residues W53α and W54α that made hydrophobic contacts with HLAα2 residues Q155 and E154 (Supplementary Fig. S3c). A summary of the contacts for both complexes are listed in supplementary Table S2.

**KRAS<sub>G12D</sub> peptide D6 side chain was buried in the HLA groove of the HLA-A*11-KRAS<sub>G12D</sub> -TCR complex**

Although there were no distinguishable differences in the interactions between the JDIa41b1 TCR and the KRAS<sup>WT</sup>/KRAS<sup>G12D</sup> peptides, there were crucial differences between the HLA-A*11-KRAS<sup>WT</sup>/KRAS<sup>G12D</sup> peptide interactions. D6 in the KRAS<sup>G12D</sup> peptide was buried in the HLA groove and formed a salt bridge with R114 in the F-pocket of the HLA, which in turn was stabilized by HLA residue D116 (Fig. 2d). The D6 side chain also made a hydrogen bond to HLA residue Q70 (Fig. 2d). These interactions were not possible with the shorter side chain of G6 in the KRAS<sup>WT</sup> peptide.

**KRAS<sub>G12D</sub> peptide D6 side chain was buried in the HLA groove of the HLA-A*11-KRAS<sub>G12D</sub> -TCR complex**

Although there were no distinguishable differences in the interactions between the JDIa41b1 TCR and the KRAS<sup>WT</sup>/KRAS<sup>G12D</sup> peptides, there were crucial differences between the HLA-A*11-KRAS<sup>WT</sup>/KRAS<sup>G12D</sup> peptide interactions. D6 in the KRAS<sup>G12D</sup> peptide was buried in the HLA groove and formed a salt bridge with R114 in the F-pocket of the HLA, which in turn was stabilized by HLA residue D116 (Fig. 2d). The D6 side chain also made a hydrogen bond to HLA residue Q70 (Fig. 2d). These interactions were not possible with the shorter side chain of G6 in the KRAS<sup>WT</sup> peptide.
versus HLA-A*11-KRASWT (PDB 7OW3), we solved the structures of both JDIα41b1 TCR bound form of each complex, a large conformational central region of the peptide (Supplementary Fig. S5d). was even more pronounced with no observable electron density in the order-like cause by peptide mobility. For HLA-A*11-KRASG12D, this was only partially observed (Supplementary Fig. S5c), suggesting dis-tron density for the central region of the peptide (residues A5 and G6) induced structures, both KRASWT and KRASG12D peptides in the pHLA structures respectively (Table 3). Unlike the conformation adopted in the TCR bound pHLAs without a bound TCR at 2.46 Å and 1.84 Å resolution, respec-tively (Table 3). Unlike the conformation adopted in the TCR bound pHLAs without a bound TCR at 2.46 Å and 1.84 Å resolution, respectively (Table 3). Unlike the conformation adopted in the TCR bound pHLAs without a bound TCR at 2.46 Å and 1.84 Å resolution, respectively (Table 3). Unlike the conformation adopted in the TCR bound pHLAs without a bound TCR at 2.46 Å and 1.84 Å resolution, respectively (Table 3). Unlike the conformation adopted in the TCR bound pHLAs without a bound TCR at 2.46 Å and 1.84 Å resolution, respectively. The remaining mutations contributed to either intra-loop or inter-loop interactions including in the TCRα-TCRβ variable interface. These mutations also potentially impacted pHLA binding through stabilisation of CDR loops and long-range network interactions. The buried surface area (BSA) on the KRASG12D peptide changed from 220 Å² (JDI TCR) to 213 Å² (JDIα41b1 TCR), and BSA on HLA changed from 770 Å² (JDI TCR) to 752 Å² (JDIα41b1 TCR), revealing marginally increased HLA surface coverage by the JDIα41b1 TCR (Supplementary Table S2).

The JDIα41b1 TCR binds to the KRASG12D peptides via an induced fit

To further investigate the mechanism underpinning the affinity window between JDIα41b1 TCR binding to HLA-A*11-KRASG12D (PDB 7OW4) versus HLA-A*11-KRASWT (PDB 7OW3), we solved the structures of both pHLAs without a bound TCR at 2.46 Å and 1.84 Å resolution, respectively (Table 3). Unlike the conformation adopted in the TCR bound pHLAs without a bound TCR at 2.46 Å and 1.84 Å resolution, respectively (Table 3). Unlike the conformation adopted in the TCR bound pHLAs without a bound TCR at 2.46 Å and 1.84 Å resolution, respectively (Table 3). Unlike the conformation adopted in the TCR bound pHLAs without a bound TCR at 2.46 Å and 1.84 Å resolution, respectively (Table 3). Unlike the conformation adopted in the TCR bound pHLAs without a bound TCR at 2.46 Å and 1.84 Å resolution, respectively (Table 3). Unlike the conformation adopted in the TCR bound pHLAs without a bound TCR at 2.46 Å and 1.84 Å resolution, respectively. The remaining mutations contributed to either intra-loop or inter-loop interactions including in the TCRα-TCRβ variable interface. These mutations also potentially impacted pHLA binding through stabilisation of CDR loops and long-range network interactions. The buried surface area (BSA) on the KRASG12D peptide changed from 220 Å² (JDI TCR) to 213 Å² (JDIα41b1 TCR), and BSA on HLA changed from 770 Å² (JDI TCR) to 752 Å² (JDIα41b1 TCR), revealing marginally increased HLA surface coverage by the JDIα41b1 TCR (Supplementary Table S2).

To further understand the key contributors of this energetic difference, we performed molecular dynamics (MD) simulations using the JDIα41b1-HLA-A*11-KRASG12D, and KRASG12D complex structures. Molecular Mechanics Poisson–Boltzmann surface area (MMPBSA) calculations were performed using 25 × 4 ns MD simulations per structure as many short repeat simulations have improved reliability compared to one long simulation25,26. The binding energies (without entropy correction) for the JDIα41b1-HLA-A*11-KRASG12D and KRASG12D complexes were determined, and the difference was calculated to be 12.6 kcal.mol⁻¹, in broad agreement with the experimentally deter-mined difference in binding enthalpy (10.8 kcal.mole⁻¹) measured in the experiment.

The JDIα41b1 TCR binds to the KRASG12D peptides via an induced fit

To further understand the key contributors of this energetic difference, we performed molecular dynamics (MD) simulations using the JDIα41b1-HLA-A*11-KRASG12D, and KRASG12D complex structures. Molecular Mechanics Poisson–Boltzmann surface area (MMPBSA) calculations were performed using 25 × 4 ns MD simulations per structure as many short repeat simulations have improved reliability compared to one long simulation25,26. The binding energies (without entropy correction) for the JDIα41b1-HLA-A*11-KRASG12D and KRASG12D complexes were determined, and the difference was calculated to be 12.6 kcal.mol⁻¹, in broad agreement with the experimentally deter-mined difference in binding enthalpy (10.8 kcal.mole⁻¹) measured in the experiment.

The JDIα41b1 TCR binds to the KRASG12D peptides via an induced fit

To further understand the key contributors of this energetic difference, we performed molecular dynamics (MD) simulations using the JDIα41b1-HLA-A*11-KRASG12D, and KRASG12D complex structures. Molecular Mechanics Poisson–Boltzmann surface area (MMPBSA) calculations were performed using 25 × 4 ns MD simulations per structure as many short repeat simulations have improved reliability compared to one long simulation25,26. The binding energies (without entropy correction) for the JDIα41b1-HLA-A*11-KRASG12D and KRASG12D complexes were determined, and the difference was calculated to be 12.6 kcal.mol⁻¹, in broad agreement with the experimentally deter-mined difference in binding enthalpy (10.8 kcal.mole⁻¹) measured in the experiment.
interactions. R28α, a residue with weak side-chain density in the JDIa41b1-HLA-A*11-KRASG12D complex, favours G12D binding, but this is fully compensated for by its HLA E58 salt-bridge partner (Supplementary Table S3). Notably, analysis of H-bonding in the MD simulations indicates that the occupancy of H-bond interactions between R28α and E58 is low, especially in the JDIa41b1-HLA-A*11-KRASG12D complex (<30%), consistent with the weak side-chain density observed.

There was a considerable difference in surface electrostatic potential of HLA-A*11-KRASG12D and -KRASWT around the mutation site, and the TCR interaction zone. This shift, towards a more negative electrostatic potential acting as a more complementary surface (Fig. 3e, f). Of the residues that were unique in the JDIa41b1 TCR compared to either the JDI wild-type TCR or the JDIa9bwt TCR (Table 4), CDR2α residue W53 (which directly contacted peptide residue Pro52 and CDR3α residue Lys94 played significant energetic roles in selectively binding to HLA-A*11-KRASG12D (Supplementary Table S3).

Overall, although the structural analysis of the TCR-pHLA complexes did not reveal an obvious mechanism for the much stronger TCR affinity for HLA-A*11-KRASG12D compared to HLA-A*11-KRASWT, the thermodynamics suggested a distinct energetic mechanism driven by a large increase in favourable electrostatic interactions, despite an increased thermodynamics suggested a distinct energetic mechanism driven by a large increase in favourable electrostatic interactions, despite an increased interaction zone. This shift, towards a more negative electrostatic potential acting as a more complementary surface (Fig. 3e, f). Of the residues that were unique in the JDIa41b1 TCR compared to either the JDI wild-type TCR or the JDIa9bwt TCR (Table 4), CDR2α residue W53 (which directly contacted peptide residue Pro52 and CDR3α residue Lys94 played significant energetic roles in selectively binding to HLA-A*11-KRASG12D (Supplementary Table S3).

Overall, although the structural analysis of the TCR-pHLA complexes did not reveal an obvious mechanism for the much stronger TCR affinity for HLA-A*11-KRASG12D compared to HLA-A*11-KRASWT, the thermodynamics suggested a distinct energetic mechanism driven by a large increase in favourable electrostatic interactions, despite an increased interaction zone. This shift, towards a more negative electrostatic potential acting as a more complementary surface (Fig. 3e, f). Of the residues that were unique in the JDIa41b1 TCR compared to either the JDI wild-type TCR or the JDIa9bwt TCR (Table 4), CDR2α residue W53 (which directly contacted peptide residue Pro52 and CDR3α residue Lys94 played significant energetic roles in selectively binding to HLA-A*11-KRASG12D (Supplementary Table S3).
energetically unfavourable shift requiring a larger disorder-order transition for complex formation. The molecular dynamics simulations demonstrated that these changes were directed by the KRAS<sup>G12D</sup> mutation, enabling energetic benefits in the TCR-pHLA interaction due to improved electrostatic complementarity between the TCR paratope and the pHLA surface.

Affinity-enhanced JDi41b1 TCR binds to KRAS<sup>G12D</sup>-pHLA with high specificity

Affinity enhancement of TCRs can alter the interface with pHLA. Ensuring that the introduced mutations maintain or even enhance selectivity, relative to other peptides in the self-repertoire, is therefore of critical importance. To expand our assessment of JDi TCR specificity further than KRAS<sup>WT</sup>, we selected a third generation affinity-enhanced JDi41b1 TCR (Fig. 1e) (HLA-A*11-KRAS<sup>G12D</sup> KD 64 ± 10 pM; HLA-A*11-KRAS<sup>WT</sup> KD 425 ± 15 nM; affinity window >6000) and panned against an HLA-A*11 pHLA library, utilising a widely adopted single chain trimer format displayed on phage to generate a peptide specificity profile<sup>29</sup> (Fig. 4a). The HLA-A*11 pHLA library encodes peptide diversity at the DNA level with approximately 6.6 × 10<sup>9</sup> variants. (Fig.4b). Following three rounds of panning, 452 unique peptides with an isolation count >1 and containing a canonical HLA-A*11 anchor (position 10 R/K) were identified and used to generate a peptide specificity profile (Fig. 4c).

Crossing angle vector is drawn connecting the disulphides between the JDi41b1 TCRα (green sphere) and TCRβ (blue sphere) variable domains. c Close up view of the JDi41b1 TCR-peptide interactions (as overlayed in a). The dotted lines indicate polar contacts. d The HLA interaction network around the peptide residue D6 in the JDi41b1-HLA-A<sup>*</sup>11-KRAS<sup>G12D</sup> complex (PBD 7OW6). e Superposition of the HLA-A<sup>*</sup>11-KRAS<sup>WT</sup> (PBD 7OW3) and HLA-A<sup>*</sup>11-KRAS<sup>G12D</sup> (PBD 7OW4) complexes without a bound TCR showing the peptides adopt open conformations. f Superposition of TCR bound JDi41b1-HLA-A<sup>*</sup>11-KRAS<sup>G12D</sup> (PBD 7OW5) and JDi41b1-HLA-A<sup>*</sup>11-KRAS<sup>WT</sup> (PBD 7OW6) complexes showing the peptides adopt closed conformations.
enriched peptides from the library parking (which accounted for 96% of all the sequenced peptides) all shared the same GADG motif at residues 4 to 7 (Table 5). Next, we sought to identify peptides in the self-repertoire that are consistent with this specificity profile and may act as structural mimetics of the KRASG12D peptide. 10 additional peptides were identified based on the amino acid preference across the full peptide length (Table 5). This panel of peptides was then used to assess JDa196b35 TCR binding to peptide-HLA-A*11 complexes other than HLA-A*11-KRASG12D using SPR. No measurable binding was detected to JDIa96b35 TCR binding to peptide-HLA-A*11 complexes other than indicating a high level of selectivity towards KRASG12D (Table 5).

In IFNγ dose-response experiments with cells presenting supra-physiological levels of peptide, IMC-KRASG12D produced half-maximal effective concentration (EC50) values of 4.6 ± 2.8 pM on cells loaded with KRASG12D peptide, consistent with the K0 values for the corresponding TCR-pHLA interaction. We also detected CD25 + CD69 + T cells in co-cultures of PBMC effectors and SUP-B15 cells incubated with decamer KRASG12D peptide and IMC-KRASG12D, but not in co-cultures lacking KRASG12D peptide, confirming T cell activation (supplementary Fig S8). In contrast, cells incubated with peptides derived from KRASWT, RASL10A & DNHD1 bound with weak affinity of 460 nM and 2.3 μM, respectively, indicating a high level of selectivity towards KRASG12D (Table 5, Supplementary Fig S7).

In order to investigate the specificity and potency of the JDI TCR in the context of a soluble T cell redirecting molecule, we generated a bispecific ImmtAC molecule consisting of the affinity-enhanced JDa196b35 TCR fused to a humanized anti-CD3-specific scFv (referred to hereafter as IMC-KRASG12D, Fig. 1e). ImmtAC molecules function by binding directly to their pHLA target presented on cancer cells, thereby activating and redirecting T cells to target tumor cells (20, 21, 22). IFNγ ELISPOT assays were used to measure the activation of unstimulated PBMC effectors when incubated with SUP-B15 cells loaded with decamer KRASG12D, KRASWT or GADG motif peptides in the presence of 100 pM IMC-KRASG12D.

Consistent with the results of the SPR experiments, significant IFNγ release was detected in co-cultures incubated with KRASG12D peptide. IFNγ release was also detected in co-cultures incubated with KRASWT peptide as well as peptides derived from the proteins encoded by RASL10A & DNHD1 containing the GADG motif, albeit at levels far below that observed for KRASG12D peptide (equivalent to approximately 1% (KRASWT and RASL10) and 12% (DNHD1) of response to KRASG12D) (Fig. 4d).

In IFNγ dose-response experiments with cells presenting supra-physiological levels of peptide, IMC-KRASG12D produced half-maximal effective concentration (EC50) values of 4.6 ± 2.8 pM on cells loaded with KRASG12D peptide, consistent with the K0 values for the corresponding TCR-pHLA interaction. We also detected CD25 + CD69 + T cells in co-cultures of PBMC effectors and SUP-B15 cells incubated with decamer KRASG12D peptide and IMC-KRASG12D, but not in co-cultures lacking KRASG12D peptide, confirming T cell activation (supplementary Fig S8). In contrast, cells incubated with peptides derived from KRASWT, RASL10A & DNHD1 bound with weak affinity of 460 nM and 2.3 μM, respectively, indicating a high level of selectivity towards KRASG12D (Table 5, Supplementary Fig S7).

In IFNγ dose-response experiments with cells presenting supra-physiological levels of peptide, IMC-KRASG12D produced half-maximal effective concentration (EC50) values of 4.6 ± 2.8 pM on cells loaded with KRASG12D peptide, consistent with the K0 values for the corresponding TCR-pHLA interaction. We also detected CD25 + CD69 + T cells in co-cultures of PBMC effectors and SUP-B15 cells incubated with decamer KRASG12D peptide and IMC-KRASG12D, but not in co-cultures lacking KRASG12D peptide, confirming T cell activation (supplementary Fig S8). In contrast, cells incubated with peptides derived from KRASWT, RASL10A & DNHD1 bound with weak affinity of 460 nM and 2.3 μM, respectively, indicating a high level of selectivity towards KRASG12D (Table 5, Supplementary Fig S7).

IMC-KRASG12D mediated T cell activation and redirected killing of cancer cells expressing KRASG12D, but not KRASWT. To further establish the epitope specificity of IMC-KRASG12D, IFNγ ELISPOT assays with professional antigen presenting cells and purified pan T cells were used to measure the activation of T cells in vitro. Immature dendritic cells (iDC) were differentiated from PBMC from patients with healthy HLA-A*11+ donors, electroporated with mRNA containing genes encoding for either KRASWT or KRASG12D, and co-cultured with autologous T cells and IMC-KRASG12D (Fig. 5a). Addition of IMC-KRASG12D to cultures resulted in a concentration dependent IFNγ release was observed from co-cultures of iDC and decamer KRASG12D. Because multiple peptides encompassing the G12 region of KRAS have been reported (20), untransfected iDC were transfected with KRASWT, RASL10A & DNHD1 containing the GADG motif, albeit at levels far below that observed for KRASG12D peptide (equivalent to approximately 1% (KRASWT and RASL10) and 12% (DNHD1) of response to KRASG12D) (Fig. 4d).
(KRAS VVGAGGVGK, VVGADGVGK, VVVGAGGVGK, VVVGADGVGK) and co-cultured with naive autologous T cells in the presence of IMC-KRASG12D. As expected, IMC-KRASG12D mediated T cell activation in co-cultures containing IDC that were pulsed with the KRASG12D decamer, but not with either of the KRASWT decamer, KRASG12D nonamer or the KRASWT nonamer peptides (Fig. 5b).

To further determine the specificity and biological activity of IMC-KRASG12D, HLA-A*11+ tumor cell lines naturally presenting KRASG12D mutations were investigated. KRASG12D mutations are present in tumors from a diverse range of lineages but are enriched in tumors as well as in cell lines derived from patients with pancreatic and colon adenocarcinoma (cancer.sanger.ac.uk). However, only a limited number of cell lines express both KRASG12D and HLA-A*11, and several of these HLA-A*11+ pancreatic KRASG12D + cell lines express only low levels of HLA or antigen processing machinery. Consistent with observations from studies of T cells modified to express murine TCR specific for KRASG12D, we only observed T cell activation in the presence of IMC-KRASG12D when these cells were modified to overexpress HLA-A*11 (see PANC-1 HLA-A*11 cells in Fig. 5e). Therefore, CRISPR/Cas9 editing was used to introduce DNA encoding either the KRASG12D mutation or HLA-A*11 into the natural locus under the control of the native promoter of a pancreatic adenocarcinoma cell line PSN-1. We confirmed the presence of DNA sequence encoding the KRASG12D mutation by sequencing the KRAS gene and used western blot analysis and specific antibodies to confirm the expression of KRASG12D (Fig. 5c), while expression of HLA-A*11 was confirmed by HLA-typing and flow cytometry with an HLA-A*11 specific antibody. The HLA- and KRAS-edited isogenic cell lines were then used as a tool to dissect the specificity of IMC-KRASG12D in a comparable and consistent cellular background. IMC-KRASG12D mediated IFNγ release was observed in ELISPOT assays using PBMC co-cultured with PSN-1 cells modified to express both KRASG12D and HLA-A*11 simultaneously (EC50 67.6 ± 15 pM & 136 ± 52 pM for clone 1 & 2 respectively), but not PSN-1 cells expressing only KRASG12D and parental HLA-A*24 (clone 4) or KRASWT and HLA-A*11 (clone 3) (Fig. 5d). Taken together, these data indicate IMC-KRASG12D is specific for KRASG12D naturally presented in the context of HLA-A*11 on the cancer cell surface.

The ability of IMC-KRASG12D to specifically mediate T cell activation and redirect T cell killing of cancer cells expressing natural KRASG12D was determined using IFNγ ELISPOT assays and a quantitative live cell imaging assay. HLA-A*11+ cancer cell lines CL40 (KRASG12D), SK-Mel-28 (KRASWT) and NCI-H2030 (KRASG12C) were utilized, as well as a panel of normal cells including pulmonary fibroblasts, cardiac myocytes, cardiac smooth muscle cells, aortic endothelial and colon epithelial cells isolated from healthy donors. Incubation of CL40 colon cancer cells with IMC-KRASG12D together with HLA-A*11+ PBMC resulted in IMC-KRASG12D concentration dependent IFNγ release in co-cultures of PBMC & HLA-A*11+ cell lines SK-Mel-28 (KRASWT), SUP-B15 (KRASWT) or NCI-H2030 (KRASG12C). Significantly, these cell lines express both KRAS and DNH1, sources of potential mimetic peptides, at physiological levels (supplementary Table S5). Incubation of KRASG12D negative cell lines with KRASG12D peptide elicited a substantial response above background in the presence of IMC-KRASG12D, confirming the integrity of these cells and their ability to present peptides (Fig. 5e). Consistent with the IFNγ ELISPOT assays, no IMC-KRASG12D mediated T cell killing was observed in co-cultures of SK-Mel-28 or NCI-H2030 with HLA-A*11+ PBMC (Supplementary Fig. S9). Most importantly, IMC-KRASG12D did not elicit IFNγ release when incubated with pulmonary, cardiac or colon-derived normal cells isolated from healthy volunteers (Fig. 5e) or result in T cell mediated targeting of normal colon epithelial cells (KRASWT), in contrast to cell lines expressing KRASG12D (Fig. 5e, f). Taken together, these data suggest that the affinity-enhanced, soluble bispecific ImmTAC molecule IMC-KRASG12D, is a potent and specific T cell engager capable of mediating T cell activation and redirected T cell targeting of cancer cells harboring KRASG12D but not cancer or normal cells expressing KRASWT.
Discussion
Recent evidence has demonstrated that it is possible to directly target cancer neoantigens via the pHLA pathway. In this study, we report the identification of a TCR that specifically recognizes a KRASG12D neoantigen peptide presented in the context of HLA-A*11. Neoantigens represent the opportunity to target cancer cells with pHLA-specific therapeutics with a low risk of off-tumor toxicity providing selectivity for the mutated epitope is retained.

Crystal structures have provided some mechanistic understanding of neoantigen specificity. The aspartate residue of the KRASG12D peptide presented by HLA-C*08:02 (GADGVGKSAL) was reported to form a salt bridge with HLA residue R156 to act as an anchor. Glycine in the wild-type peptide at position 3 is not an optimal anchor residue and hence is not effectively presented by HLA-C*08:02. In this case, poor wild-type peptide presentation was likely the mechanism underlying TCR specificity towards KRASG12D mutant peptide. A similar mechanism, whereby somatic mutations introduce preferred primary HLA anchor residues, has been shown to generate public, shared neoantigens, for example in PIK3CA mutations encoding PI3Kα H1047L and histone variant H3.3K27M. In contrast, both KRASWT and KRASG12D peptides used in this study had comparable predicted binding affinities to HLA-A*11, suggesting that selectivity based on peptide presentation was unlikely to occur. Despite this, biochemical analysis of the JDI TCR demonstrated a preference for KRASG12D and it was possible to enhance the binding affinity of this TCR to HLA-A*11-KRASG12D by over a million-fold, while conserving the ability to distinguish between KRASWT and KRASG12D.

Structural analysis of an affinity-enhanced version of the JDI TCR in complex with HLA-A*11-KRASWT and -KRASG12D demonstrated a virtually identical interaction network. However, analysis of the pHLA crystal structures revealed that both peptides underwent an induced fit conformational change upon TCR binding, with peptide residue D6 in the HLA-A*11-KRASG12D complex forming a network of stabilizing interactions with the HLA-A*11 surface. This change in peptide conformation was reflected by thermodynamic analysis, demonstrating that TCR binding to HLA-A*11-KRASG12D was driven by a greater net formation of new electrostatic interactions, at the cost of a greater disorder-order transition compared to HLA-A*11-KRASWT. Further analysis using molecular dynamics simulations suggested that the buried D6 residue mediated a change towards negative electrostatics on the HLA surface, increasing the electrostatic interactions with the TCR. This allowed energetically favourable electrostatic interactions, in part mediated by TCR residues R50 and K70, that formed an indirect network of interactions with the pHLA surface.

Table 5 | Mimetic peptides selected from the human genome based on the binding motif determined using scHLA phage library screening

| UniProtID | Gene | Peptide amino acid residue number: | 1-10 Peptide Score | Motif Score | NetMICpan4.1 | Ko (M) |
|-----------|------|-----------------------------------|-------------------|------------|-------------|--------|
| P01116    | KRASG12D | V V V G A D G V G K | 0.67 | 0.92 | 194.20 | 6.40E-11 |
| P01116    | KRASWT   | V V V G A D G V G K | 0.58 | 0.69 | 153.55 | 4.30E-07 |
| Q9628     | PREL1D3A | S V L G V D V L Q R | 0.68 | 0.53 | 132.59 | -     |
| Q76LX8    | ADA1TMS13 | S V S C G D G I Q R | 0.66 | 0.61 | 567.49 | -     |
| P58304    | VSX2     | T V S G P D S L A R | 0.65 | 0.65 | 333.05 | -     |
| Q92737    | RASL10A  | A V L G A P G V G K | 0.63 | 0.73 | 23.04 | 4.60E-07 |
| O14974    | PPP1R12A | T V T S A A G L Q K | 0.63 | 0.51 | 44.08 | -     |
| O95238    | SPDEF    | A A A G A V G L E R | 0.63 | 0.69 | 625.61 | -     |
| Q9628     | DNHD1    | T V L G P N G V G K | 0.63 | 0.65 | 31.32 | 2.30E-06 |
| P17987    | TCP1     | S S L G P V G L D K | 0.62 | 0.64 | 18.46 | -     |
| P46643    | CCT5     | T S L G P N G L D K | 0.62 | 0.65 | 84.28 | -     |
| Q5D086    | DALRD3   | T V L V A D H L A R | 0.62 | 0.50 | 603.65 | -     |
the EGFR\textsuperscript{S442H} mutation that was clinically associated with acquired cetuximab resistance in colorectal cancers\textsuperscript{39}. These findings add further to our understanding of how TCRs can use indirect energetically driven mechanisms to sense differences not only in peptide sequence, but also in peptide dynamics, that can drive T cell antigen specificity\textsuperscript{42}. The affinity enhanced TCR bispecific protein tebentafusp (CD3 + gp100), has been shown to redirect polyclonal T cells differentiated from monocytes isolated from healthy donors were transfected with mRNA encoding KRAS\textsuperscript{WT} or KRAS\textsuperscript{G12D} or b pulsed with indicated exogenous peptide, were treated with IMC-KRAS\textsuperscript{G12D} and co-cultured with autologous T cells for 24 h. T cell activation was measured by IFN\gamma ELISPOT assay. Three biological replicates from one representative experiment from two independent experiments are shown. c Immunoblot and densitometry analysis of isogenic PSN-1 cell lysates. Blot represents triplicate of two independent experiments. Uncropped blots can be viewed in Supplementary Fig. S10. d lmIFN ELISPOT output of cell lines modified to express HLA-A*11 and KRAS\textsuperscript{G12D} (clones 1 & 2), HLA-A*11 and KRAS\textsuperscript{WT} (clone 3) or only KRAS\textsuperscript{G12D} (clone 4) treated with IMC-KRAS\textsuperscript{G12D} and co-cultured with PBMC for 24 h. Targets alone, or no IMC-KRAS\textsuperscript{G12D} negative controls (Cont.) were performed. Mean data of three biological replicates ±SEM from one representative experiment from three independent experiments is shown. e IFN\gamma ELISPOT output of an extended panel of HLA-A*11-KRAS\textsuperscript{G12D}+ and HLA-A*11-KRAS\textsuperscript{WT}, cancer cell lines and healthy cells, treated with IMC-KRAS\textsuperscript{G12D} and co-cultured with PBMC for 24 h. HLA-A*11 + /KRAS\textsuperscript{G12D} cell lines were pulsed with 10\textmu M KRAS\textsuperscript{WT} (+G12D) peptide as a positive control. Mean data of three biological replicates ±SEM from one representative experiment from two independent experiments is shown f. Redirected T cell killing assay of HLA-A*11 + /KRAS\textsuperscript{G12D}. CL40 cancer or HLA-A*11 + /KRAS\textsuperscript{WT} normal human colon epithelial cells expressing nuclear-restricted mKATE2, treated with IMC-KRAS\textsuperscript{G12D} and co-cultured with PBMC for 72 h. Cells were pulsed with 10\textmu M KRAS\textsuperscript{WT} (+G12D) peptide as a positive control. Targets alone, PBMC alone or no IMC-KRAS\textsuperscript{G12D} negative controls (Cont.) were performed. Mean data of three biological replicates ±SD from one representative experiment from two independent experiments is shown. Source data are provided as a Source Data file.

the EGFR\textsuperscript{S442H} mutation that was clinically associated with acquired cetuximab resistance in colorectal cancers\textsuperscript{39}. These findings add further to our understanding of how TCRs can use indirect energetically driven mechanisms to sense differences not only in peptide sequence, but also in peptide dynamics, that can drive T cell antigen specificity\textsuperscript{42}. The affinity enhanced TCR bispecific protein tebentafusp (CD3 + gp100), has been shown to redirect polyclonal T cells differentiated from monocytes isolated from healthy donors were transfected with mRNA encoding KRAS\textsuperscript{WT} or KRAS\textsuperscript{G12D} or b pulsed with indicated exogenous peptide, were treated with IMC-KRAS\textsuperscript{G12D} and co-cultured with autologous T cells for 24 h. T cell activation was measured by IFN\gamma ELISPOT assay. Three biological replicates from one representative experiment from two independent experiments are shown. c Immunoblot and densitometry analysis of isogenic PSN-1 cell lysates. Blot represents triplicate of two independent experiments. Uncropped blots can be viewed in Supplementary Fig. S10. d lmIFN ELISPOT output of cell lines modified to express HLA-A*11 and KRAS\textsuperscript{G12D} (clones 1 & 2), HLA-A*11 and KRAS\textsuperscript{WT} (clone 3) or only KRAS\textsuperscript{G12D} (clone 4) treated with IMC-KRAS\textsuperscript{G12D} and co-cultured with PBMC for 24 h. Targets alone, or no IMC-KRAS\textsuperscript{G12D} negative controls (Cont.) were performed. Mean data of three biological replicates ±SEM from one representative experiment from three independent experiments is shown. e IFN\gamma ELISPOT output of an extended panel of HLA-A*11-KRAS\textsuperscript{G12D}+ and HLA-A*11-KRAS\textsuperscript{WT}, cancer cell lines and healthy cells, treated with IMC-KRAS\textsuperscript{G12D} and co-cultured with PBMC for 24 h. HLA-A*11 + /KRAS\textsuperscript{G12D} cell lines were pulsed with 10\textmu M KRAS\textsuperscript{WT} (+G12D) peptide as a positive control. Mean data of three biological replicates ±SEM from one representative experiment from two independent experiments is shown f. Redirected T cell killing assay of HLA-A*11 + /KRAS\textsuperscript{G12D}. CL40 cancer or HLA-A*11 + /KRAS\textsuperscript{WT} normal human colon epithelial cells expressing nuclear-restricted mKATE2, treated with IMC-KRAS\textsuperscript{G12D} and co-cultured with PBMC for 72 h. Cells were pulsed with 10\textmu M KRAS\textsuperscript{WT} (+G12D) peptide as a positive control. Targets alone, PBMC alone or no IMC-KRAS\textsuperscript{G12D} negative controls (Cont.) were performed. Mean data of three biological replicates ±SD from one representative experiment from two independent experiments is shown. Source data are provided as a Source Data file.

the EGFR\textsuperscript{S442H} mutation that was clinically associated with acquired cetuximab resistance in colorectal cancers\textsuperscript{39}. These findings add further to our understanding of how TCRs can use indirect energetically driven mechanisms to sense differences not only in peptide sequence, but also in peptide dynamics, that can drive T cell antigen specificity\textsuperscript{42}. The affinity enhanced TCR bispecific protein tebentafusp (CD3 + gp100), has been shown to redirect polyclonal T cells differentiated from monocytes isolated from healthy donors were transfected with mRNA encoding KRAS\textsuperscript{WT} or KRAS\textsuperscript{G12D} or b pulsed with indicated exogenous peptide, were treated with IMC-KRAS\textsuperscript{G12D} and co-cultured with autologous T cells for 24 h. T cell activation was measured by IFN\gamma ELISPOT assay. Three biological replicates from one representative experiment from two independent experiments are shown. c Immunoblot and densitometry analysis of isogenic PSN-1 cell lysates. Blot represents triplicate of two independent experiments. Uncropped blots can be viewed in Supplementary Fig. S10. d lmIFN ELISPOT output of cell lines modified to express HLA-A*11 and KRAS\textsuperscript{G12D} (clones 1 & 2), HLA-A*11 and KRAS\textsuperscript{WT} (clone 3) or only KRAS\textsuperscript{G12D} (clone 4) treated with IMC-KRAS\textsuperscript{G12D} and co-cultured with PBMC for 24 h. Targets alone, or no IMC-KRAS\textsuperscript{G12D} negative controls (Cont.) were performed. Mean data of three biological replicates ±SEM from one representative experiment from three independent experiments is shown. e IFN\gamma ELISPOT output of an extended panel of HLA-A*11-KRAS\textsuperscript{G12D}+ and HLA-A*11-KRAS\textsuperscript{WT}, cancer cell lines and healthy cells, treated with IMC-KRAS\textsuperscript{G12D} and co-cultured with PBMC for 24 h. HLA-A*11 + /KRAS\textsuperscript{G12D} cell lines were pulsed with 10\textmu M KRAS\textsuperscript{WT} (+G12D) peptide as a positive control. Mean data of three biological replicates ±SEM from one representative experiment from two independent experiments is shown f. Redirected T cell killing assay of HLA-A*11 + /KRAS\textsuperscript{G12D}. CL40 cancer or HLA-A*11 + /KRAS\textsuperscript{WT} normal human colon epithelial cells expressing nuclear-restricted mKATE2, treated with IMC-KRAS\textsuperscript{G12D} and co-cultured with PBMC for 72 h. Cells were pulsed with 10\textmu M KRAS\textsuperscript{WT} (+G12D) peptide as a positive control. Targets alone, PBMC alone or no IMC-KRAS\textsuperscript{G12D} negative controls (Cont.) were performed. Mean data of three biological replicates ±SD from one representative experiment from two independent experiments is shown. Source data are provided as a Source Data file.
of TCR therapeutics to target neoantigens and have important implications for understanding the molecular determinants of therapeutic TCR selectivity for neoantigens.

**Methods**

**Primary Cell and cell line culture, antibodies and reagents**

Cell lines and normal cells were purchased from the suppliers listed in supplementary table S6 and cultured in media recommended by the supplier. KRAS (exon 2 region) was sequenced from PCR products amplified from gDNA isolated using QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) or prepared from healthy donor leukopaks (ALLCELLS, CA) by Ficoll density gradient centrifugation. CD14 + monocyte cells isolated by negative selection from PBMCs using Classical Monocyte Isolation Kit (Miltenyi, North Rhine-Westphalia, Germany) were differentiated for 4–6 days in Gibco™ AIM V Medium (Thermo Fisher Scientific, MA) with Penicillin-Streptomycin (Gibco™), 5% AB serum (Valley Biomedical, VA), 1000 U/ml GM-CSF and 300 U/ml IL-4 (Bio-technne, MN) to generate iDC. Where relevant, T cells were isolated from PBMC by immunopurification using a Pan T Cell Isolation Kit (Miltenyi) according to the manufacturer’s instructions. Rabbit anti-RAS, −RASG12D and HRP-conjugated secondary antibodies were purchased from Cell Signaling Technology (Danvers, MA). Anti-GAPDH was purchased from Merck Millipore (MA). Purified synthetic peptides were purchased from Peptide Protein Research Ltd, UK.

**TCR receptor isolation, affinity maturation and protein production**

A TCR specific to HLA-A11-KRASG12D was isolated from a healthy donor using established methods: T cells were isolated from a HLA-A0201/ A11+ donor and stimulated with 7 days with autologous dendritic cells that had been pulsed with 1 µM KRASG12D peptide (VVVGADGVGK) to displace naturally presented peptides. For this, the dendritic cells were incubated with exogenous peptide for two hours, then washed two times with culture media. T cells were subsequently stimulated twice more over an additional 14-day period with autologous activated B cells that had been pulsed with 0.1 µM KRASG12D peptide. T cell cultures were screened by IFNγ ELISPOT assay to identify KRASG12D peptide specific T cells. T cell clones were sorted on the basis of the expression of the activation markers CD25 and CD37 after incubation with the KRASG12D peptide by using a FACSARia (BD Biosciences). TCR gene sequences were identified from a specific T cell clone by rapid amplification of cDNA ends (RACE).

TCR affinity maturation was performed using phage display methodology. Briefly, NKN primer driven mutations were introduced into CDR segments of TCRα and TCRβ genes and phage libraries were constructed by overlap extension PCR. Wild-type HLA-A11-KRAS was used for negative selection of phage libraries prior to selection on HLA-A11-KRASG12D to facilitate identification of mutant peptide selective clones. Three rounds of selection were performed with decreasing antigen concentration to enrich clones with enhanced affinity. Mutants were screened using competitive inhibition ELISA to select specific affinity enhanced clones. To produce soluble disulfide-linked mTCRs and IMC-KRASG12D molecules, TCRα and TCRβ chain inclusion bodies were denatured using 50 mM Tris buffer pH 8.1 containing 100 mM Sodium Chloride, 6 M Guanidine and 20 mM Dithiothreitol (DTT) and refolded by diluting to final protein concentration of 60 mg/ml into a buffer containing 100 mM Tris pH 8.1, 4 M Urea, 400 mM L-Arginine, 1.9 mM Cystamine and 6.5 mM Cysteamine redox reagent. Refolding mixture was dialysed against water followed by 20 mM Tris buffer pH 8.1. Correctly folded protein was purified by anion exchange chromatography, cation exchange chromatography and size exclusion as previously described.

**Binding affinity and thermodynamic parameter measurement**

phLA complexes were refolded as previously reported. The HLA heavy chain AviTag was biotinylated using BirA500 biotin-protein ligase kit (Avityde LLC, USA). Biotinylated phHLAs were immobilized on a Streptavidin-coated CMS sensor chip using a Biacore T200/Biacore 8 K (Cytiva life sciences, USA). 400–500 RU of biotinylated phLA was immobilized for multi cycle steady state analysis of weak affinity TCRs using Biacore T200 and 100-200 RU of phLA for single cycle kinetic analysis of strong affinity TCRs using Biacore 8 K. 5000 RU of common peptide (CP) pool phLA was used for TCR cross reactivity testing. JDI TCR affinity was measured by 10-point steady state analysis using TCR concentration ranging from 50 µM to 0.1 µM. Alanine scan of JDI TCR was performed using a 10 point titration using TCR concentration ranging from 100 µM to 0.2 µM. Multi cycle steady state equilibrium analysis data was processed using one site total least squares fit in GraphPad Prism 9.0. Single cycle kinetic analysis of high affinity TCRs was performed using 5-point titration with top concentration at least 50x higher than Kd. Single cycle kinetic data was processed using Biacore Insight Evaluation software. A Minimum of 2 measurements were performed to obtain Kd values reported for JDI TCR, JDIa9bwt, JDIa41b1 and IMC-KRASG12D. Binding affinities were measured (n = 2) at 6 °C intervals from 6 °C-36 °C using a Biacore T200 for thermodynamic parameter determination. Data was analyzed using the Thermodynamics wizard in Biacore T200 Evaluation Software 3.1. Non-linear van’t Hoff plots were used to determine enthalpy (ΔH), entropy (ΔS) and Gibbs free energy (ΔG).

**Crystallisation and protein structure determination**

The TCR-phLA complexes were prepared by mixing purified TCR and phLa at a molar ratio of 1:1:5. The TCR-phLa and phLA samples were concentrated to ~10 mg/ml and crystallisation trials were set up by dispensing 150 nL of protein solution plus 150 nL of reservoir solution in sitting-drop vapor diffusion format in two-well MCC crystallization plates using a Gryphon robot (Art Robbins Instruments, LLC). Plates were maintained at 20 °C in a Rock Imager 10000 (Formulatrix) storage system. Diffraction quality crystals for HLA-A11-KRASWT and HLA-A11-KRASG12D complexes were grown in the following conditions: 0.2 M Ammonium sulfate, 0.1 M Sodium cacodylate pH 6.0, 25% PEG 4000; and 0.2 M Lithium sulfate, 0.1 M Bis-Tris pH 5.5, 25% PEG 3550, respectively. Diffraction quality crystals for JDI TCR-HLA-A11-KRASG12D, JDIa41b1HLA-A11-KRASWT and JDIa41b1-HLA-A11-KRASG12D complexes were grown in the following conditions: 0.2 M Ammonium sulfate, 0.1 M Tris pH 8.5, 20% PEG 8000; 0.2 M Sodium nitrate, 20% PEG 3350; and 0.2 M Ammonium sulfate, 20% PEG 3350, respectively. X-ray diffraction data for the four samples were collected at the Diamond Light Source (Oxfordshire, UK) beamlines (HLA-A11-KRASWT – DLS 103; HLA-A11-KRASG12D – DLS 104; JDIa41b1HLA-A11-KRASWT and JDIa41b1-HLA-A11-KRASG12D – DLS 104). Diffraction images for the HLA-A11-KRASWT and HLA-A11-KRASG12D complexes were indexed, integrated, scaled, and merged using the autoproc pipeline implementing XDS/XSCALE and POINTLESS/AIMLESS. Diffraction images for the JDI TCR-HLA-A11-KRASG12D, JDIa41b1HLA-A11-KRASWT and JDIa41b1-HLA-A11-KRASG12D complexes were indexed, integrated, scaled, and merged using the xia2 automated pipeline implementing DIALS. Structures were solved by molecular replacement using PDB 4UQ2 for HLA and β2m, and a hybrid TCR generated by combining PDB 4 JRX (TCR α chain) and PDB 4X6B (TCR β chain) as search models in Phaser. Two separate PDBs were chosen for the TCR α and β chains as these individually have very high sequence identity (>90%) for the JDI TCR chains. The TCR β chain from PDB 4X6B was superposed onto and in the place of CAS TCR beta chain (PDB 4JRX) to create the hybrid TCR search model. Models were built using iterative cycles of manual model building in COOT56 and refinement using Refmac in the CCP4 suite. The stereochemical properties and validation of the models were assessed using the PDB-REDO and Molprobity.
The data processing and refinement statistics are listed in Table 3. Structural figures were prepared using PyMOL (Schrödinger, LLC).

**Molecular dynamic simulations-MMPBSA Calculations**

The following is a short overview of the computational methods used herein; a detailed description is provided in the Supplementary Materials. The X-ray structures were used as starting points for MD simulations in aqueous solvent with a 100 mM sodium chloride concentration. Prior to production simulations, minimization, heating, and equilibration was performed as described in Supplementary Materials. The first 1 ns of the production MD was discarded to allow for further equilibration, meaning 25 × 3 ns of simulation and a total of 7500 snapshots were used in each MMPBSA calculation. The MMPBSA.py MPI script was used on Amber1661,62. A salt concentration of 100 mM was used (with the default dielectric). All ions were stripped from the trajectories, prior to MMPBSA calculation, and the closest 30 explicit waters to the binding interface were retained. Differences in the contribution to the binding energy were calculated as the average WT value subtracted from the average GI2D value at each residue. Multiple T-tests were performed on GraphPad Prism 9 (GraphPad Software, CA) to determine the significance of the differences at the P < 0.01 level of significance.

**Generation of HLA-A*11 scHLA libraries**

Peptide HLA libraries were generated in a single chain format with peptide-b2m- HLA-A*11 displayed on the surface of phase as disulfide trapped single chain trimers (dsSCT). Briefly a randomized 10-mer peptide library consisting of 1 × 10^10 peptide diversity was synthesized (Twist Biosciences, USA) and cloned into a phagemid scHLA construct using a pelB leader sequence and C-terminal coat protein pIII21. This phagemid library was introduced by electroporation into E. coli TG1 cells with KM13 helper phage to enable monoclonal display with an estimated library size of 6.6 × 10^10 colonies22. Diversity was confirmed post electroporation by next generation sequencing of the initial library. This confirmed that all 20 amino acids were represented at every position with a flat distribution of 5% (max 5.8%, min 4.5%).

**Panning of scHLA libraries with JDL4a1b1 TCR**

Streptavidin-coated paramagnetic beads M280 (Thermo Fisher Scientific, USA) were saturated with biotinylated TCR and phage selections performed as described previously23. Colonies were scraped and phagemid purified by miniprep (Qiagen). Sequencing libraries were prepared by amplification of the peptide region. Purified PCR products (Ampure XP beads, Beckman coulter) were prepared using NebNext Ultra II DNA library prep kit (NEB, E7645S) and 2 × 150 bp paired end read amplicon sequencing performed with the Illumina MiSeq. From >0.9 million peptides sequences 96.5% had an R/K as the preferred read amplicon sequencing performed with the Illumina MiSeq. From Ultra II DNA library prep kit (NEB, E7645S) and 2 × 150 bp paired end (Ampure XP beads, Beckman coulter) were prepared using NebNext (Sartorius) equipped with a ×10 objective over 96 h at 2 h intervals. Target cell lysis, normalized to control co-cultures without IMC-KRASG12D and expressed as % cytolyis, was detected using the red object metric in the IncuCyte software, and analyzed with Prism 8 software (Graphpad Software).

**CRISPR/Cas9 editing**

**IFNy ELISPOT assay**

IFNy ELISPOT assays were performed using BD™ ELISPOT reagents in accordance with manufacturer’s instructions66. For assays using exogenous peptide loaded cells, target cells were incubated with 10 µm peptide (unless otherwise indicated) and co-cultured with PBMC from non-HLA*A11:01/03:01 donors in the presence of IMC-KRASG12D in RPMI-1640 medium containing 25 mM HEPES and supplemented with Penicillin-Streptomycin (Gibco®) and 10% fetal bovine serum overnight at 37 °C. For assays using IDC, capped mRNA encoding KRASG12D (TriLink, CA) was electroporated into iDCs using Amasa Human B Cell Nucleofector Kit (Lonza Switzerland) with a single electrical pulse on Amasa Nucleofector II device (Lonza) using program X-00L. The dendritic cells were allowed to recover in complete AIM-V medium at 37 °C overnight and were then harvested and co-cultured with T cells. Alternatively, IDCs were pulsed with 5 µg/ml peptides (Bio-Synth, TX) for 1 h at 37 °C, and then co-cultured with T cells. Freshly isolated T cells were then co-cultured with iDCs at a 5:1 ratio and treated with IMC-KRASG12D in CTL test medium (CTL) overnight at 37 °C on the 96-well Strip Precoated Human IFNy Single-Color Enzymatic ELISPOT plate (CTL). IFNy release was quantified using the the CTL ImmunoSpot Analyzer and ImmunoSpot® software. (ImmunoSpot Series 5 Analyzer, CTL). Redirected T cell killing assays Kinetic redirected T cell killing assays were performed using live cell imaging24. Briefly, target cells were transduced with commercially available lentivirus encoding mKATE2 modified to restrict expression to the nucleus. Co-cultures of mKATE2-expressing target cells and PBMC effector cells (Effector: Target ratio 10:1) were seeded onto ImageLock well plates (Sartorius, Göttingen, Germany) and treated with IMC-KRASG12D in the presence of NuclView™ Caspase-3/7 apotosis assay reagent (Sartorius). Images were acquired using an IncuCyte Zoom (Sartorius) equipped with a ×10 objective over 96 h at 2 h intervals. Target cell lysis, normalized to control co-cultures without IMC-KRASG12D and expressed as % cytolyis, was detected using the red object metric in the IncuCyte software, and analyzed with Prism 8 software (Graphpad Software).
First, using a pool of two HDR templates [one to mutate KRAS in PSN-1 and introduce a non-targetable (G/R)12G mutation (introducing silent mutations at the guide target site)] and the second to introduce a G12D mutation (G12D repair template: GTATACCCCTTGCTGGATCATGTCCTGGCCTCCTGGCGGACGCAAGCTCAGTGAGCTACCTTACGATATTAGGAATTAGACTGTGCTGTGGGCGGCATGGCGTAGGCAAGAGTGCGTTGACGATACAGCTAATTCAGAATCATTTTGTGGACGAATATGATCCAACAATAGAGG TAAATCTTGTTTTAAT]. The first step resulted in the isolation of 3 PSN-1 clones with KRAS(R/G)12G and the second step transfecting a pool of the PSN-1 KRAS(R/G)12G clones with the KRAS guide and G12D repair template resulted in 2 clones that were validated by western blot and gDNA sequencing.

HLA-A allele conversion was achieved using an HDR template consisting of two HLA-A*24 homology arms flanking a HLA-A*11 core region that would transcribe and translate a functional HLA-A*11 protein. The left and right homology arms were PCR amplified from the genomic DNA of PSN-1 and covered exons 1 and 6 of HLA-A*24, respectively, while the DNA fragment covering exons 2-5 of HLA-A*11 core was PCR amplified from genomic DNA of CL40 cells. The HLA-A*24 homology arms and HLA-A*11 core fragments were stitched by PCR and the amplified product was used as a HDR template for the HLA-A conversion of PSN-1 clones. HLA-A allele conversion of selected clones was validated by HLA-typing performed by VH Bio Ltd (UK).

Western blot analysis
Cells were lysed in RIPA buffer (Thermo Fisher Scientific) containing protease and phosphatase inhibitors (Sigma-Aldrich, MO), separated using SDS-PAGE and transferred onto nitrocellulose membranes. Antibodies were diluted as described in supplementary table S7 in TBS containing 0.1% Tween-20 and 1% milk or BSA. Bound HRP secondary antibodies were detected and quantified on western blots using a C-digit scanner and Image Studio software (Li-Cor, NE).

Reporting summary
Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
The SPR and cell-based assay data generated in this study are provided in the Source Data file. The crystallography data generated in this study have been deposited in the RCSB protein data bank (PDB) with the accession codes 7OW3, 7OW4, 7OW5, 7OW6 and 7PB2. Starting structures, parameters and input files for the molecular dynamics simulations and MMPBSA calculations are available via https://doi.org/10.3821/zenodo.6839257 Source data are provided with this paper.

References
1. Yarchoan, M., Johnson, B. A., Lutz, E. R., Laheru, D. A. & Jaffee, E. M. Targeting neoantigens to augment antitumour immunity. Nat. Rev. Cancer 17, 209–222 (2017).
2. Schumacher, T. N. & Schreiber, R. D. Neoantigens in cancer immunotherapy. Science 348, 69–74 (2015).
3. Douglass, J. et al. Bispecific antibodies targeting mutant RAS neoantigens. Sci. Immunol. 6, eaab5515 (2021).
4. Hsieu, E. H.-C. et al. Targeting a neoantigen derived from a common TP53 mutation. Science 371, eabc6897 (2021).
5. Tran, E. et al. Immunogenicity of somatic mutations in human gastrointestinal cancers. Science 350, 1387–1390 (2015).
6. Cafri, G. et al. Memory T cells targeting oncogenic mutations detected in peripheral blood of epithelial cancer patients. Nat. Commun. 10, 449 (2019).
7. Castle, J. C., Udumian, M., Pabla, S., Stein, R. B. & Buell, J. S. Mutation-derived neoantigens for cancer immunotherapy. Front. Immunol. https://doi.org/10.3389/fimmu.2019.01856 (2019).
8. Chen, F. et al. Neoantigen identification strategies enable personalized immunotherapy in refractory solid tumors. J. Clin. Investig. 129, 2056–2070 (2019).
9. Prior, I. A., Lewis, P. D. & Mattos, C. A comprehensive survey of rs mutations in cancer. Cancer Res. 72, 2457–2467 (2012).
10. Downward, J. Targeting RAS signalling pathways in cancer therapy. Nat. Rev. Cancer 3, 11–22 (2003).
11. Lu, S., Jiang, H., Nussinov, R. & Zhang, J. The structural basis of oncopgenic mutations G12, G13 and Q61 in small GTPase K-Ras4B. Sci. Rep. 6, 21949 (2016).
12. Tuveson, D. A. et al. Endogenous oncogenic K-ras (G12D) stimulates proliferation and widespread neoplastic and developmental defects. Cancer Cell 5, 375–387 (2004).
13. Prior, I. A., Hood, F. E. & Hartley, J. L. The frequency of rs mutations in cancer. Cancer Res. 80, 2969–2974 (2020).
14. Chen, H., Smaill, J. B., Liu, T., Ding, K. & Lu, X. Small-molecule inhibitors directly targeting KRAS as anticancer therapeutics. J. Med. Chem. 63, 14424–14424 (2020).
15. Hong, D. S. et al. KRAS(G12C) inhibition with sotorasib in advanced solid tumors. N. Engl. J. Med. 383, 1207–1217 (2020).
16. Canon, J. et al. The clinical KRAS(G12C) inhibitor AMG 510 drives anti-tumour immunity. Nature 575, 217–223 (2019).
17. Fell, J. B. et al. Identification of the clinical development candidate MRTX849, a covalent KRAS(G12C) inhibitor for the treatment of cancer. J. Med. Chem. 63, 6679–6693 (2020).
18. Chatani, P. D. & Yang, J. C. Mutated RAS: targeting the "untearable" with T cells. Clin. Cancer Res. 26, S37–S44 (2020).
19. Tran, E. et al. T-cell transfer therapy targeting mutant KRAS in cancer. N. Engl. J. Med. 375, 2255–2262 (2016).
20. Wang, Q. J. et al. Identification of T-cell Receptors Targeting KRAS-Mutated Human Tumors. Cancer Immunol. Res. 4, 204–214 (2016).
21. Lowe, K. L. et al. Novel TCR-based biologics: mobilising T cells to warm ‘cold’ tumours. Cancer Treat. Rev. 77, 35–43 (2019).
22. Liddy, N. et al. Monoclonal TCR-redirected tumor cell killing. Nat. Med. 18, 980–987 (2012).
23. Li, Y. et al. Directed evolution of human T-cell receptors with picomolar affinity by phage display. Nat. Biotechnol. 23, 349–354 (2005).
24. Dunn, S. M. et al. Directed evolution of human T cell receptor CD2 residues by phage display dramatically enhances affinity for cognate peptide-MHC without increasing apparent cross-reactivity. Protein Sci. 15, 710–721 (2006).
25. Madura, F. et al. T-cell receptor specificity maintained by altered thermodynamics *. J. Biol. Chem. 288, 18766–18775 (2013).
26. Crean, R. M. et al. Molecular rules underpinning enhanced affinity binding of human T cell receptors engineered for immunotherapy. Mol. Ther. Oncolytics 18, 443–456 (2020).
27. Holland, C. J. et al. Specificity of bispecific T cell receptors and antibodies targeting peptide-HLA. J. Clin. Investig. 130, 2673–2688 (2020).
28. Genheden, S. & Ryde, U. The MM/PBSA and MM/GBSA methods to estimate ligand-binding affinities. Expert Opin. Drug Discov. 10, 449–461 (2015).
29. Mitakos, V. et al. Structural engineering of pMHC reagents for T cell vaccines and diagnostics. Chem. Biol. 14, 909–922 (2007).
30. Middleton, M. R. et al. Tebentafusp, A TCR/anti-CD3 bispecific fusion protein targeting gp100, potently activated antitumor
immune responses in patients with metastatic melanoma. Clin. Cancer Res. 26, 5869–5878 (2020).

31. Tate, J. G. et al. COSMIC: the catalogue of somatic mutations in cancer. Nucleic Acids Res. 47, D941–D947 (2019).

32. Scholtalbers, J. et al. TCLP: an online cancer cell line catalogue integrating HLA type, predicted neo-epitopes, virus and gene expression. Genome Med. 7, 118 (2015).

33. Sim, M. J. W. et al. High-affinity oligoclonal TCRs define effective adoptive T cell therapy targeting mutant KRAS-G12D. Proc. Natl. Acad. Sci. USA 117, 12826–12835 (2020).

34. Bai, P. et al. Immune-based mutation classification enables neoantigen prioritization and immune feature discovery in cancer immunotherapy. Oncoimmunology 10, 1868130 (2021).

35. Chandran, S. S. et al. Immunogenicity of a public neoantigen derived from mutated PIK3CA. bioRxiv https://doi.org/10.1101.2021.04.08.439061 (2021).

36. Chheda, Z. S. et al. Novel and shared neoantigen derived from histone 3 variant H3.3K27M mutation for glioma T cell therapy. J. Exp. Med. 215, 141–157 (2017).

37. Coles, C. H. et al. T cell receptor interactions with human leukocyte antigen govern indirect peptide selectivity for the cancer testis antigen MAGE-A4. J. Biol. Chem. 295, 11486–11494 (2020).

38. Madura, F. et al. TCR-induced alteration of primary MHC peptide anchor residue. Eur. J. Immunol. 49, 1052–1066 (2019).

39. Bianchi, V. et al. A molecular switch abrogates glycoprotein 100 (gp100) T-cell receptor (TCR) targeting of a human melanoma antigen*. J. Biol. Chem. 291, 8951–8959 (2016).

40. Madura, F. et al. Structural basis for ineffective T-cell responses to MHC anchor residue-improved “heteroclitic” peptides. Eur. J. Immunol. 45, 584–591 (2015).

41. Bagchi, A. et al. Molecular basis for necitumumab inhibition of EGFR variants associated with acquired cetuximab resistance. Mol. Cancer Ther. 17, 521–531 (2018).

42. Hopkins, J. R. et al. Peptide cargo tunes a network of correlated motions in human leucocyte antigens. FEBS J. 287, 3771–3793 (2020).

43. Nathan, P. et al. Overall survival benefit with tebentafusp in metastatic uveal melanoma. N. Engl. J. Med. 385, 1196–1206 (2021).

44. Cameron et al. Identification of a trin-derived HLA-A1–presented peptide as a cross-reactive target for engineered MAGE A3–directed T cells. Sci. Transl. Med. 5, 1971ra103 (2013).

45. Wu, D., Gallagher, D. T., Gowthaman, R., Pierce, B. G. & Mariuzza, R. A. Structural basis for oligoclonal T cell recognition of a shared p53 cancer neoantigen. Nat. Commun. 11, 2908 (2020).

46. Riley, T. P. et al. Structure based prediction of neoantigen immunogenicity. Front. Immunol. https://doi.org/10.3389/fimmu.2019.02047 (2019).

47. Laugel, B. et al. Different T cell receptor affinity thresholds and CD8 coreceptor dependence govern cytotoxic T lymphocyte activation and tetramer binding properties. J. Biol. Chem. 282, 3799–3810 (2007).

48. Boulter, J. M. et al. Stable, soluble T-cell receptor molecules for crystallization and therapeutics. Protein Eng., Des. Sel. 16, 707–711 (2003).

49. Garboczi, D. N., Hung, D. T. & Wiley, D. C. HLA-A2-peptide complexes: refolding and crystallization of molecules expressed in Escherichia coli and complexed with single antigenic peptides. Proc. Natl. Acad. Sci. USA 89, 3429–3433 (1992).

50. Vonhein, C. et al. Data processing and analysis with the autoPROC toolbox. Acta Crystallogr. Sect. D 67, 293–302 (2011).

51. Kabach, W. XDS. Acta Crystallogr. D Biol. Crystallogr. 66, 125–132 (2010).

52. Evans, P. R. & Murshudov, G. N. How good are my data and what is the resolution. Acta Crystallogr. D Biol. Crystallogr. 69, 1204–1214 (2013).

53. Winter, G. xia2: an expert system for macromolecular crystallography data reduction. J. Appl. Crystallogr. 43, 186–190 (2010).

54. Winter, G. et al. DIALS: implementation and evaluation of a new integration package. Acta Crystallogr. Sect. D 74, 85–97 (2018).

55. McCoy, A. J. et al. Phaser crystallographic software. J. Appl Crystallogr. 40, 658–674 (2007).

56. Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. Features and development of Coot. Acta Crystallogr. D Biol. Crystallogr. 66, 486–501 (2010).

57. Murshudov, G. N. et al. REFMAC5 for the refinement of macromolecular crystal structures. Acta Crystallogr. Sect. D 67, 355–367 (2011).

58. Winn, M. D. et al. Overview of the CCP4 suite and current developments. Acta Crystallogr. D Biol. Crystallogr. 67, 235–242 (2011).

59. Joosten, R. P., Joosten, K., Murshudov, G. N. & Perrakis, A. PDB_REDO: constructive validation, more than just looking for errors. Acta Crystallogr. D Biol. Crystallogr. 68, 484–496 (2012).

60. Williams, C. J. et al. MolProbity: more and better reference data for improved all-atom structure validation. Protein Sci. 27, 293–315 (2018).

61. Miller, B. R. et al. MMPBSA.py: an efficient program for end-state free energy calculations. J. Chem. Theory Comput. 8, 3314–3321 (2012).

62. D.A. Case et al. Amber 2021. (University of California, 2021).

63. Lee, C. M. Y., Iorno, N., Sierro, F. & Christ, D. Selection of human antibody fragments by phage display. Nat. Protoc. 2, 3001–3008 (2007).

64. The UniProt C. UniProt: the universal protein knowledgebase in 2021. Nucleic Acids Res. 49, D480–D489 (2021).

65. Reynisson, B., Alvarez, B., Paul, S., Peters, B. & Nielsen, M. NetMHCpan-4.1 and NetMHCclip-4.0: improved predictions of MHC antigen presentation by concurrent motif deconvolution and integration of MS MHC eluted ligand data. Nucleic Acids Res. 48, W449–W454 (2020).

66. Harper, J. et al. An approved in vitro approach to preclinical safety and efficacy evaluation of engineered T cell receptor anti-CD3 bispecific (ImmTAC) molecules. PLoS ONE 13, 1–19, e0205491 (2018).

67. Dobrzynki, T., Ciuantu, A., Stacey, A., Dukes, J. D. & Whale, A. D. in Immuno-Oncology: Cellular and Translational Approaches (ed Seng-Lai Tan) 51–72 (Springer US, 2020).

Acknowledgements

We would like to thank Michelle McCully, Laury Humbert, Peter Molloy, and Ita O’Kelly for critical reading and help preparing this manuscript. We also wish to thank: Vanessa Clarke, Max Beckmann, Sarah Bailey, Nicole Mai, Tein Foong-Leong, and Peter James for discussions and supporting identification of the KRASG12D specific TCR and affinity maturation; Kathryn Lamming, Alex Powlesland, and Ricardo Carreira for cell line characterisation; Hemza Ghadbane for design of CRISPR guides, repair templates and editing approach used to edit HLA and KRAS alleles, and Carmine Carpenito and JoAnn Suzich for their scientific input and insights. We would like to thank Diamond Light Source and staff of beamlines for access to beamtime (proposal mx22870). A.H. and M.v.d.K acknowledge for design of CRISPR guides, repair templates and editing approach used to edit HLA and KRAS alleles, and Carmine Carpenito and JoAnn Suzich for their scientific input and insights. We would like to thank Diamond Light Source and staff of beamlines for access to beamtime (proposal mx22870). A.H. and M.v.d.K thank the Engineering and Physical Sciences Research Council [grant number EP/TS17872/1] and the Biotechnology and Biological Sciences Research Council [grant number BB/M026280/1] for support. Computer simulations were conducted using the computational facilities of the Advanced Computing Research Centre of the University of Bristol.

Author contributions

A.P., V.K., A.H., S.M., T.D., K.B., M.H., C.H., C.R.J.D., C.Co., W.Y., and A.D.W. performed experiments and data analyses. J.N.H., S.H., R.R.,
A.B.L., J.D.D., M.A., N.L., M.v.d.K, G.D.P., A.V., D.K.C., A.D.W., and C.C. designed experiments and supervised the project. V.K., A.H., M.v.d.K, D.K.C., A.D.W., and C.C. wrote the manuscript.

**Competing interests**

A.P., V.K., S.M., T.D., C.H., C.R., J.D., S.H., K.B., M.H., C.C., M.A., R.R., J.D.D., N.L., A.V., D.K.C., A.D.W., and C.C. are current or former employees of, and may hold shares in Immunocore Ltd. J.N.H, W.Y., A.B.L., and G.D.P. are current or former employees of, and may hold shares in Eli Lilly and Co. M.v.d.K and A.H. declare no competing interests.

**Additional information**

**Supplementary information** The online version contains supplementary material available at https://doi.org/10.1038/s41467-022-32811-1.

**Correspondence** and requests for materials should be addressed to Andrew D. Whale or Chandramouli Chillakuri.

**Peer review information** Nature Communications thanks Brian Baker and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available.

**Reprints and permission information** is available at http://www.nature.com/reprints

**Publisher’s note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2022