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Supplementary Information for:

High affinity oligoclonal TCRs define effective adoptive T-cell therapy targeting mutant KRAS-G12D

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Figure S1.

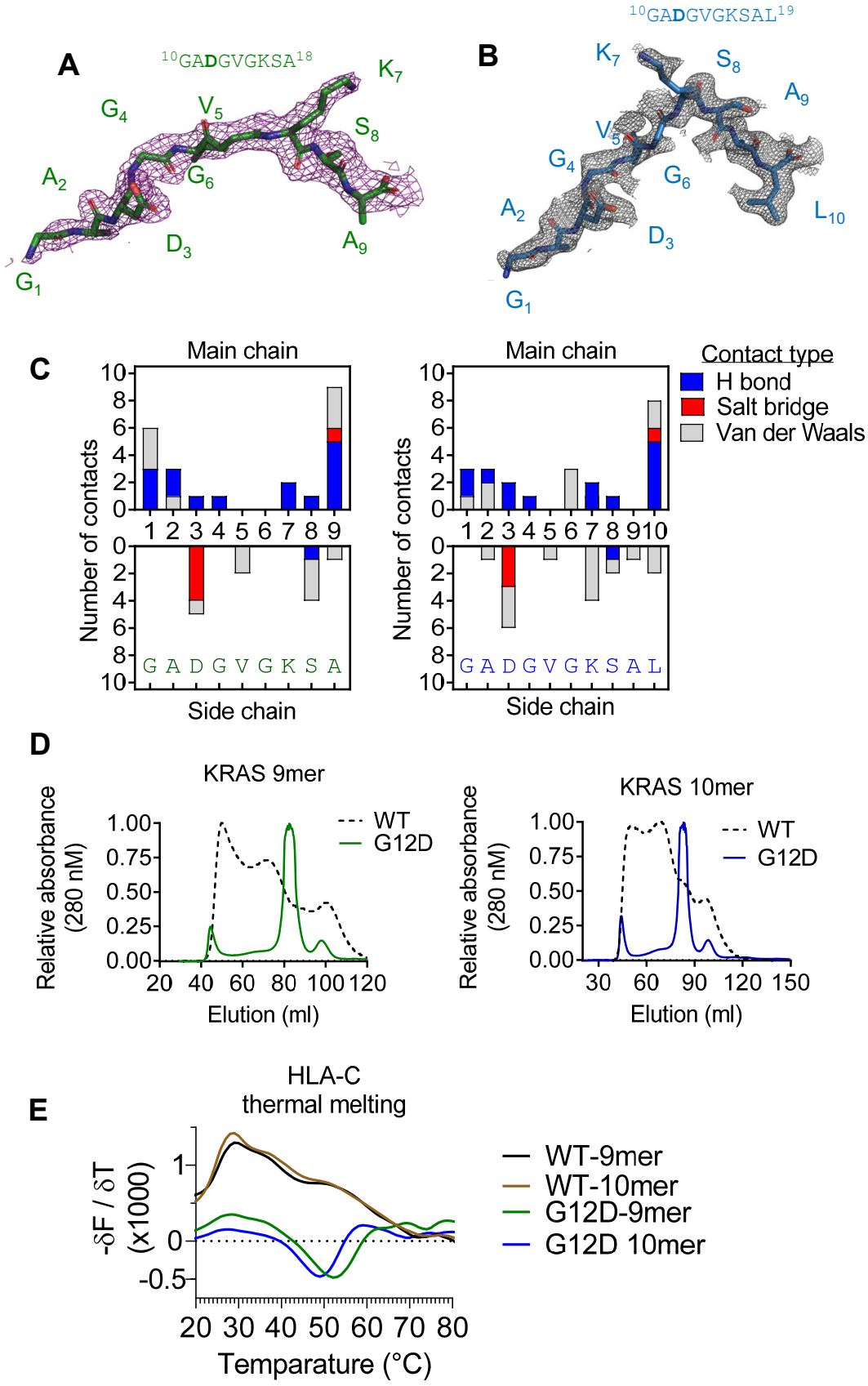
A
10GADGVGKSA18
K7
G4
V5
S8
A2
G6
D3
A9
G1
B
10GADGVGKSAL19
K7
S8
A9
V5
G4
G6
A2
L10
D3
G1
C
Main chain
Number of contacts
Side chain
G A D G V G K S A
Main chain
Number of contacts
Side chain
G A D G V G K S A L
Contact type
H bond
Salt bridge
Van der Waals
D
KRAS 9mer
Relative absorbance (280 nM)
Elution (ml)
WT
G12D
KRAS 10mer
Relative absorbance (280 nM)
Elution (ml)
WT
G12D
E
HLA-C
thermal melting
-δF / δT (x1000)
Temparature (°C)
WT-9mer
WT-10mer
G12D-9mer
G12D 10mer

Figure S1.
(A-B) Stick model of the KRAS-G12D 9mer (A) and 10mer (B) bound to HLA-C*08:02 with 2Fo-Fc omit maps contoured to 1.2σ.
(C) Number of contacts between HLA-C*08:02 and the KRAS-G12D 9mer (*left*) and 10mer (*right*). Contacts with the peptide main chain (*top)* and side chain (*bottom*) residues. Contacts are defined as hydrogen (H) bonds, salt bridges or van der Waals contacts between atoms within 3.5 Å.
(D) Frequency of TCR^+ Jurkat T cells expressing CD69 after incubation with 221-C*08:02-ICP47 cells loaded with WT, G12D, G12V, G12R, and G12S KRAS 9mer and 10mer peptides. Peptides were tested from 1000 nM to 1 nM, shown here at 100 nM, data are a summary of 2 independent experiments. Statistical significance was assessed by one-way ANOVA with Dunnett's multiple comparison test (*** $p<0.001$,**** $p<0.0001$).

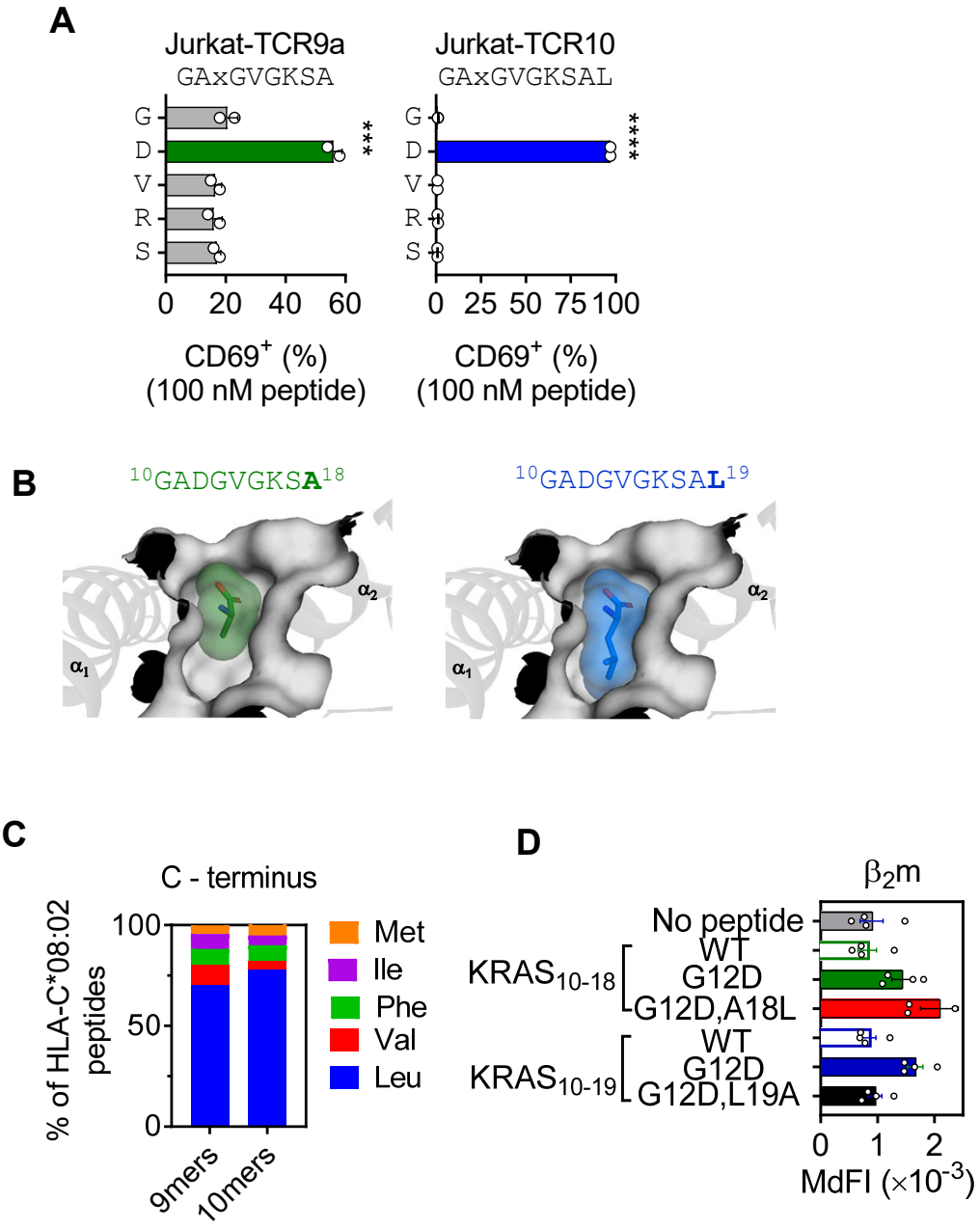

Figure S2.
(A) Traces of size exclusion chromatography for HLA-C*08:02 refolded with WT or G12D KRAS 9mer (left) or 10mer (right) peptides. Traces are normalized to the maximum absorbance (280 nm) for each experiment.
(B) Space filling model of the C-terminal residue of the G12D 9mer (left) and 10mer (right).
(C) Amino acid frequency at the C-terminus of 9mer and 10mer peptides eluted from HLA-C*08:02. Data from (22).
(D) HLA-I stabilization on 221-C*08:02-ICP47 cells incubated overnight at 26°C with 100 μM wild type (WT) and G12D KRAS 9mer, 10mer and C-terminal modified peptides. Data are a summary of 4 independent experiments.

Figure S3.

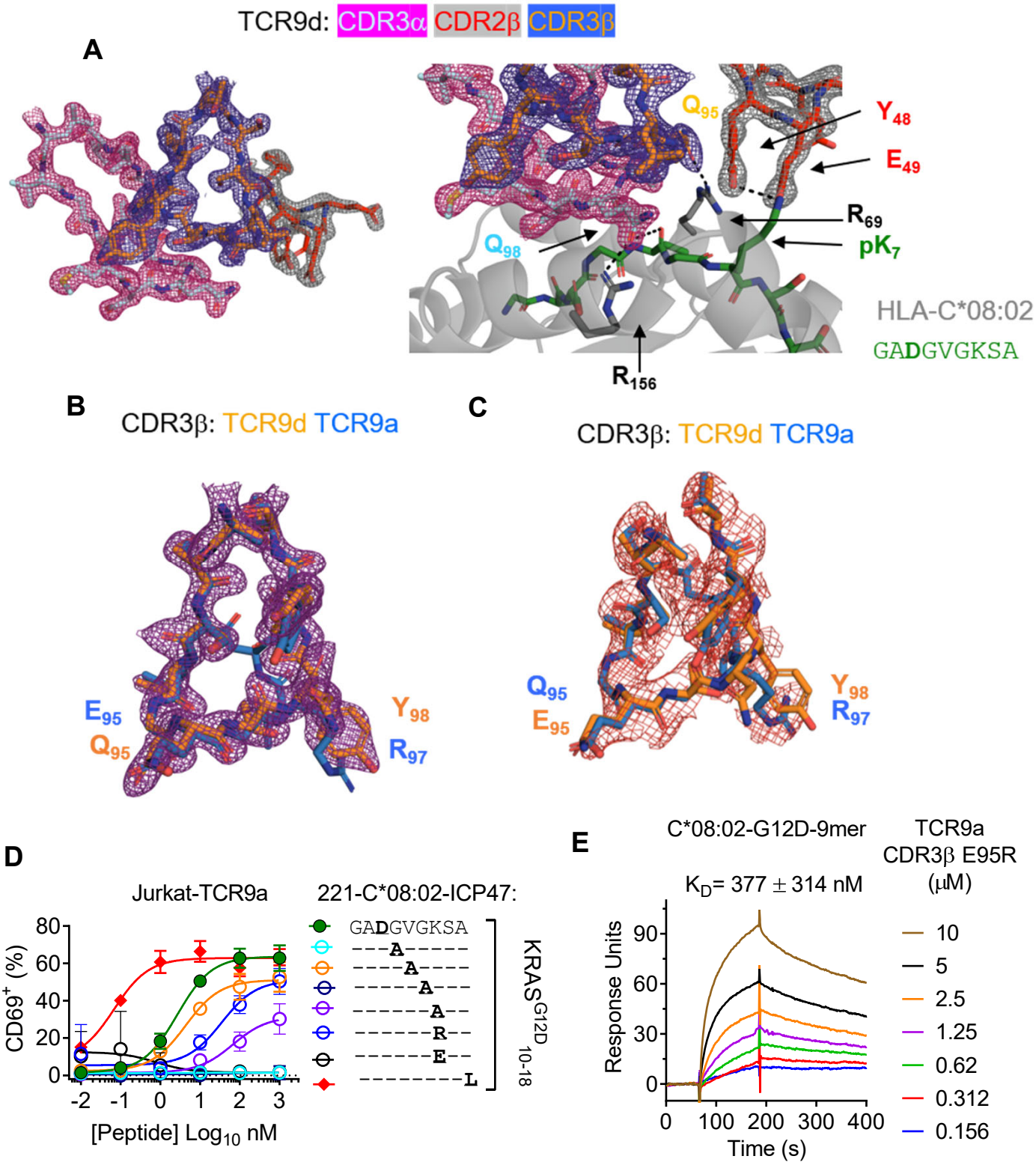
TCR9d: CDR3α CDR2β CDR3β
A
Q95
Y48
E49
R69
pK7
Q98
R156
HLA-C*08:02
GADGVGKSA
B
CDR3β: TCR9d TCR9a
E95
Q95
Y98
R97
C
CDR3β: TCR9d TCR9a
Q95
E95
Y98
R97
D
Jurkat-TCR9a
221-C*08:02-ICP47:
GADGVGKSA
---A-----
----A----
-----A---
------A--
------R--
------E--
--------L
KRASG12D 10-18
CD69+ (%)
[Peptide] Log10 nM
E
C*08:02-G12D-9mer
KD= 377 ± 314 nM
TCR9a CDR3β E95R (μM)
Response Units
Time (s)
10
5
2.5
1.25
0.62
0.312
0.156

Figure S3.
(A) 2Fo-Fc omit map of TCR9d contoured to 2σ. CDR3α, CDR3β and CDR2β, are shown alone (left) and in complex with HLA-C (right). CDR3α, turquoise; CDR3β orange; CDR2β, red; HLA-C, grey; KRAS-G12D-9mer, green.
(B-C) Comparing CDR3β structures of TCR9d and TCR9a. 2Fo-Fc omit map of TCR9d contoured to 2σ (B) and TCR9a 2Fo-Fc omit map of TCR9d contoured to 1.2σ (C). CDR3β contoured to 1σ. CDR3β TCR9d, orange; map purple, CDR3β TCR9a, blue; map orange.
(D) Frequency of TCR^+ Jurkat T cells expressing CD69 after incubation with 221-C*08:02-ICP47 cells loaded with WT and G12D KRAS 9mer and mutant 9mer peptides. Amino acids identical to the KRAS sequence are indicated with –. Peptides were tested from 1 nM to 0.1 nM. Data are a mean of 3-4 independent experiments.
(E) Binding of TCR9a-CDR3β E95R to immobilized HLA-C*08:02-KRAS-G12D-9mer at indicated μM concentrations determined by surface plasmon resonance (SPR). Dissociation constants were determined by kinetic curve fitting. Data are representative of two independent experiments.

Figure S4.

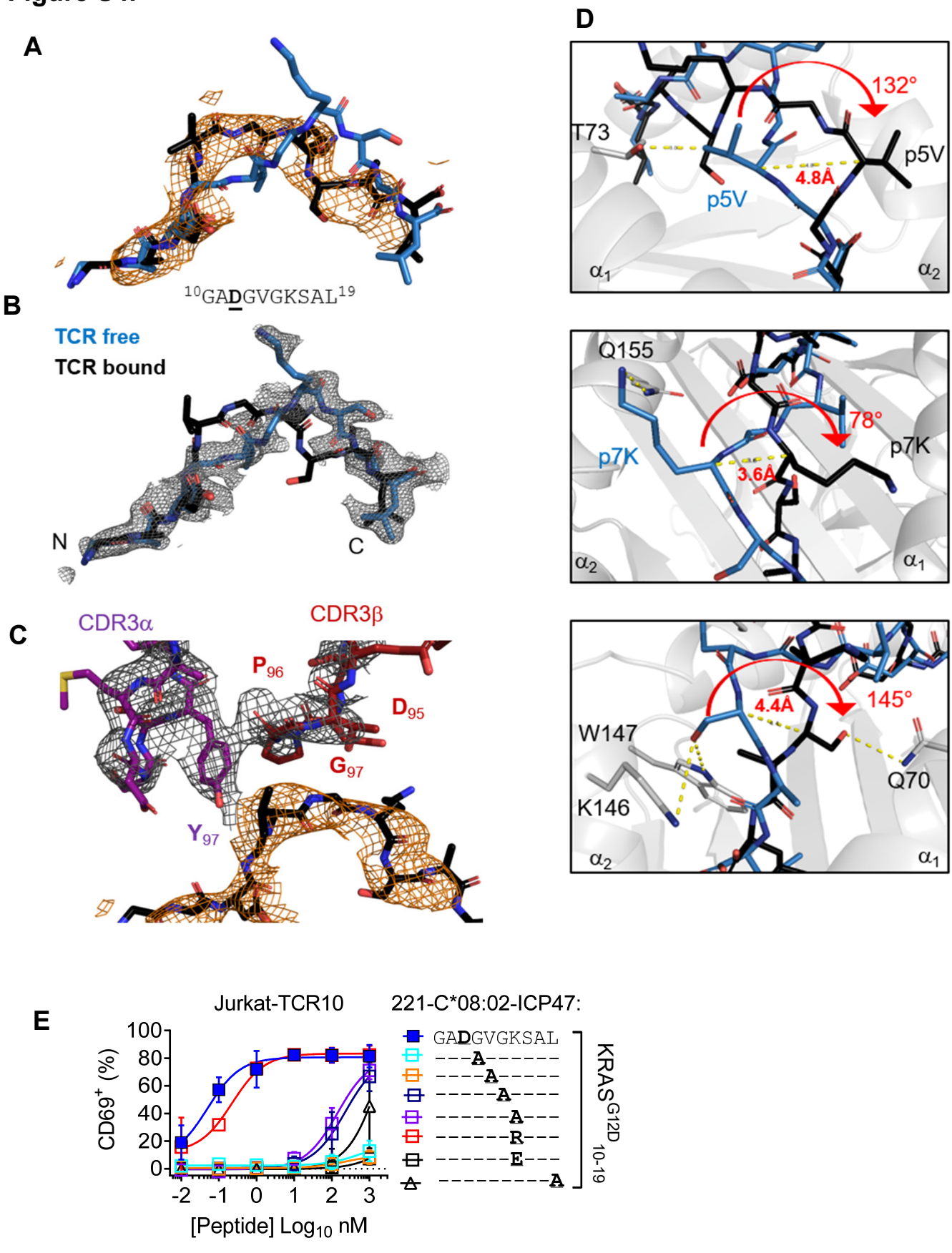
A
10GADGVGKSAL19
B
TCR free
TCR bound
N
C
C
CDR3α
CDR3β
P96
D95
G97
Y97
D
132°
T73
p5V
4.8Å
p5V
α1
α2
Q155
78°
p7K
p7K
3.6Å
α2
α1
4.4Å
145°
W147
Q70
K146
α2
α1
E
Jurkat-TCR10
221-C*08:02-ICP47:
CD69+ (%)
100
80
60
40
20
0
-2 -1 0 1 2 3
[Peptide] Log10 nM
GADGVGKSAL
---A------
----A-----
-----A----
------A---
------R---
------E---
---------A
KRASG12D 10-19

Figure S4.
(A) 2Fo-Fc omit map 'TCR free' KRAS-G12D 10mer bound to HLA-C*08:02, contoured to 1σ. 'TCR free' model, blue; map, orange; 'TCR10 bound' model, black; HLA-C, grey.
(B) 2Fo-Fc omit map map of 'TCR bound' KRAS-G12D 10mer bound to HLA-C*08:02, contoured to 1.2σ. 'TCR bound' model, black; map, red; 'TCR10 free' model, blue; HLA-C, grey.
(C) 2Fo-Fc omit map of TCR10 KRAS-G12D 10mer bound to HLA-C*08:02, contoured to 1σ. CDR3α, purple; CDR3β, red; KRAS-G12D-10mer, black; HLA-C, grey.
(D) Changes in the KRAS-G12D 10mer between the TCR free (blue) and TCR bound conformations (black). (*Top*) Cα of Val (p5) shifted by 4.8 Å and rotated by 132° breaking a van der Waals (VDW) contact with Thr 73 in the α_1 helix of HLA-C*08:02. (Middle) The Lys at (p7) shifted 3.6 Å and rotated by 78° towards TCR10 breaking h-bonds with Gln 155 α_2 helix of HLA-C*08:02. (Bottom) Ser (p8) shifted 4.4 Å and rotated 145° breaking with h-bonds Lys 146 with Trp 147 in the α_2 helix of HLA-C*08:02 and forming a new h-bond with Gln 70 in the α_1 helix of HLA-C*08:02.
(E) Frequency of TCR^+ Jurkat T cells expressing CD69 after incubation with 221-C*08:02-ICP47 cells loaded with WT and G12D KRAS 10mer and mutant 10mer peptides. Amino acids identical to the KRAS sequence are indicated with –. Peptides were tested from 1nM to 0.1 nM. Data are a mean of 3-4 independent experiments.

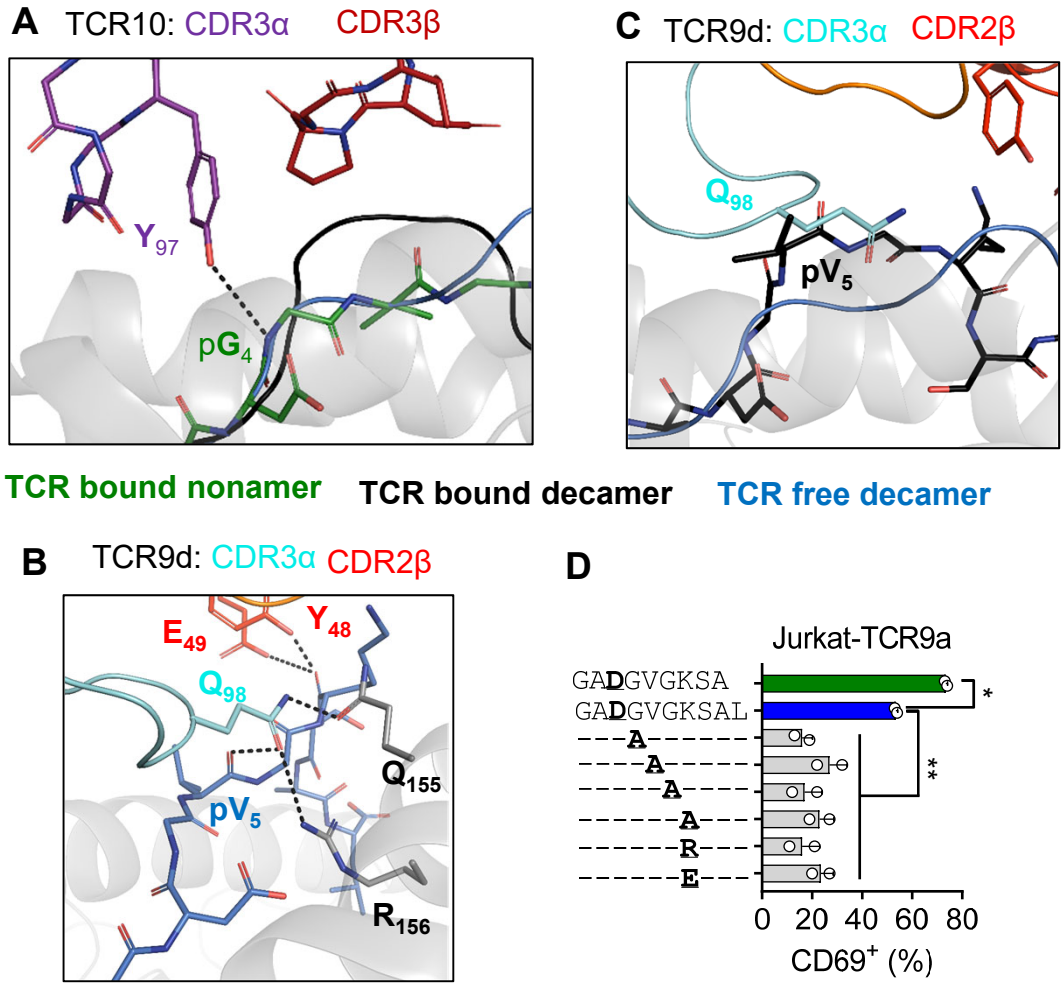

Figure S5.
(A) Modelling of TCR10 CDR3α interaction with KRAS-G12D 9mer. CDR3α, purple; CDR3β red; HLA-C, grey; KRAS-G12D-9mer, green; KRAS-G12D-10mer TCR bound, black; KRAS-G12D-10mer TCR free, blue.
(B) Modelling of TCR9d CDR3α and CDR3β interactions with KRAS-G12D 10mer in TCR bound conformation. CDR3α, turquoise; CDR3β orange; HLA-C, grey; TCR bound, black; KRAS-G12D-10mer TCR free, blue.
(C) Modelling of TCR9d CDR3α and CDR3β interactions with KRAS-G12D 10mer in TCR free conformation. CDR3α, turquoise; CDR3β orange; HLA-C, grey; KRAS-G12D-10mer TCR free, blue.
(D) Frequency of TCR$^+$ Jurkat T cells expressing CD69 after incubation with 221-C*08:02-ICP47 cells loaded with KRAS-G12D 9mer, and 10mer peptides with indicated amino acid substitutions. Amino acids identical to the KRAS sequence are indicated with –. Peptides were tested from 1000 nM to 1 nM, shown here at 100 nM, data are a summary of 2 independent experiments. Statistical significance was assessed by one-way ANOVA with Dunnett's multiple comparison test (* $p<0.05$, ** $p<0.01$).

	HLA-C*08:02-GADGVGKSA	HLA-C*08:02-GADGVGKSAL	TCR9d HLA-C*08:02-GADGVGKSA	TCR9a-HLA-C*08:02-GADGVGKSA	TCR10-HLA-C*08:02-GADGVGKSAL
PDB code	6ULI	6ULK	6ULN	6ULR	6UON
Data collection					
Temperature (K)	100.00	100.00	100.00	100.00	100.00
Space group	C 1 2 1	C 1 2 1	P1 2_1 1	P1 2_1 1	P1 2_1 1
Cell dimensions					
a, b, c (Å)	95.3, 76.1, 62.7	95.6, 77.1, 62.3	72.8, 74.0, 107.5	73.0, 74.0, 107.9	63.4, 79.3, 196.7
α, β, γ (°)	90.0, 118.1, 90.0	90.0, 120.6, 90.0	90.0, 101.7, 90.0	90.0, 101.5, 90.0	90.0, 91.1, 90.0
Resolution range (Å)	50.0-1.9 (1.90-1.93)	50.0-1.9 (1.90-1.93)	50.0-2.0 (2.00-2.03)	50.0-3.2 (3.20-3.26)	50.0-3.5 (3.5-3.56)
R_{merge} (%)	9.5 (63.2)	11.2 (39.5)	15.2 (85.9)	17.4 (48.8)	28 (111)
I/σ(I)	42.1 (4.0)	21.8 (8.7)	21.5 (2.0)	25.0 (9.3)	16.7 (1.9)
Completeness (%)	100 (100)	99.5 (100)	100 (99.4)	95.9 (92.3)	100 (100)
Redundancy	7 (6.1)	5.3 (6.4)	7 (5.1)	5.4 (5.3)	8.9 (7.0)
Total observations	217185	118478	526630	96239	237149
Unique observations	31888 (1534)	30790 (1574)	75046 (3708)	17905 (874)	26510 (1293)
Refinement					
Refinement resolution (Å)					
R_{work} *(%)*	19.9	22.1	18.5	22.2	25.2
R_{free} *(%)*	22.6	27.0	21.8	26.2	29.4
No. of atoms					
Protein	3123	3089	6485	6485	13108
Water	128	229	545	30	0
Mean B-factor ($Å^2$)	44.10	31.00	38.40	42.60	126.08
rmsd from ideal values					
Bond lengths (Å)	0.006	0.004	0.004	0.002	0.005
Bond angles (°)	0.82	0.7	0.60	0.51	0.89
Ramachandran statistics					
Favored	97.90	95.23	95.62	93.37	95.02
Outliers	0.00	0.00	0.00	0.00	0.00

Table S1. Structural data and refinement statistics.
Data for outer shell shown in parentheses ().

TCR	T_m (ºC)
9a	47.3 ± 0.4
9b	47 ± 0
9c	45.9 ± 2.0
9d	46.8 ± 0.4
10	61.8 ± 0.4

Table S2. Melting temperatures of recombinant KRAS-G12D specific TCRs.

	Vα4	HLA-C	Vα10
	3	E58	
	25	R62	14
	8	K66	3
	3	R69	
		R131	6
		E154	3
	10	Q155	4
	3	R156	
		R157	1
		A158	3
	3	T163	3
	13	E166	
	11	W167	
	1	R169	
	1	R170	
Total	**81**		**37**
	Vα4	**Peptide**	**Vα10**
	2	pG4	8
	11	pV5	1
	5	pG6	
Total	**18**		**9**
	Vβ5	**HLA-C**	**Vβ12**
		Q65	1
	12	R69	8
	2	A150	
	1	R151	
	4	Q155	1
Total	**19**		**10**
	Vβ5	**Peptide**	**Vβ12**
		pG4	2
		pV5	9
		pG6	1
	9	pK7	5
Total	**9**		**17**

Table S3. TCR contacts with peptide-HLA-C. Number of contacts with each TCR chain and either HLA-C or peptide**.** Data for complexes of TCR9d (6ULN) and TCR10 (6UON) with HLA-C.

	HLA-C	Peptide	%
Vα4	81	18	78.0
Vβ5	19	9	22.0
%	78.7	21.3	

	HLA-C	Peptide	%
Vα10	37	9	63.0
Vβ12	10	17	37.0
%	64.4	35.6	

Table S4. Number of TCR contacts with peptide or HLA-C. Data for complexes of TCR9d (6ULN) and TCR10 (6UON) with HLA-C.