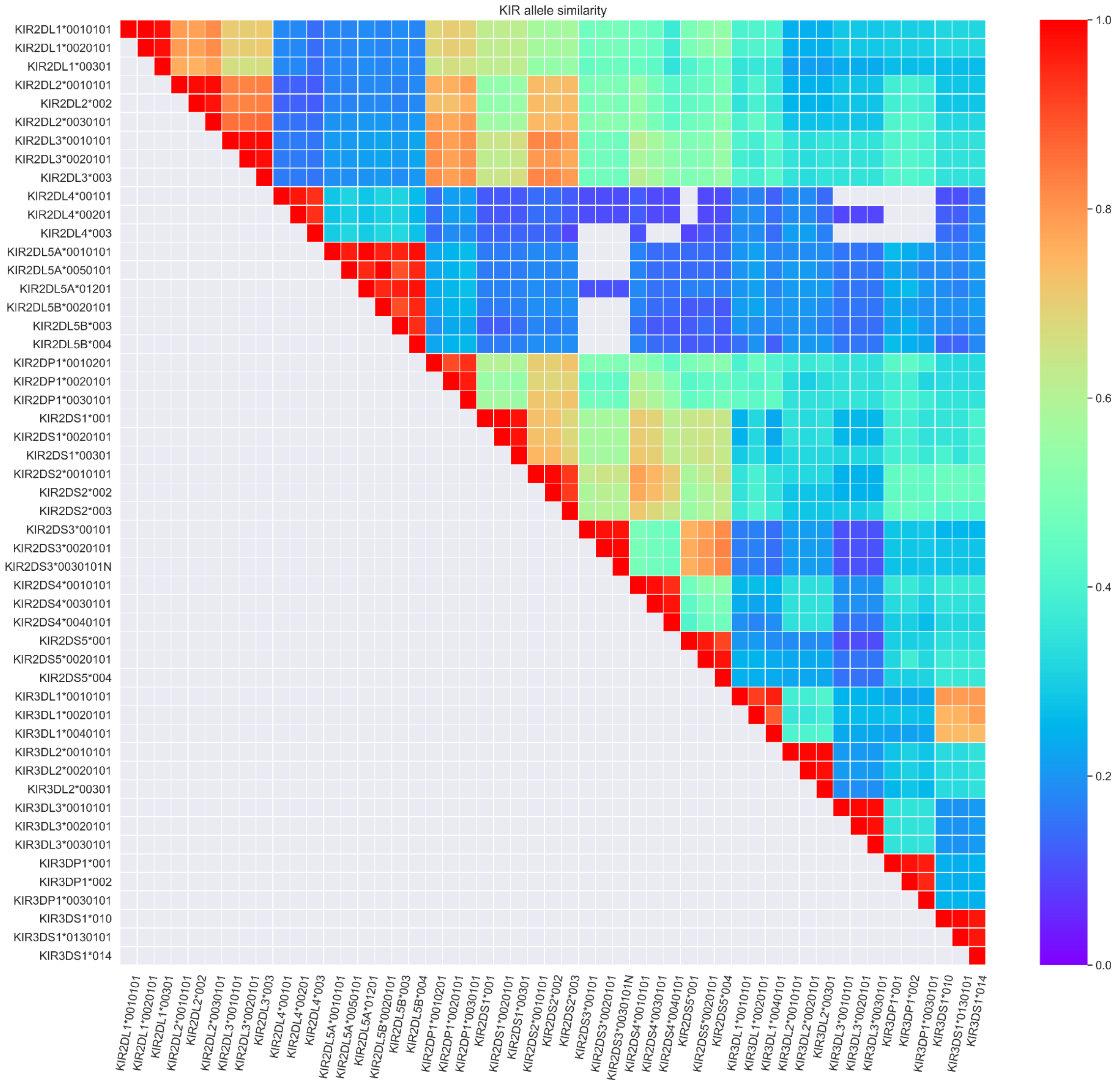
Figure S1: Allele similarity of the mRNA sequences extracted from IPD-KIR and IPD-IMGT/HLA database. Each gene has up to three representative alleles in the heatmap. The color shows the sequence similarity estimated from minimap2 (with -x ava-pb). Only upper triangular portion is colored, and the grey color in upper triangle indicates that minimap2 can not find good alignment between two sequences.

(a) KIR allele similarity



(b) HLA allele similarity

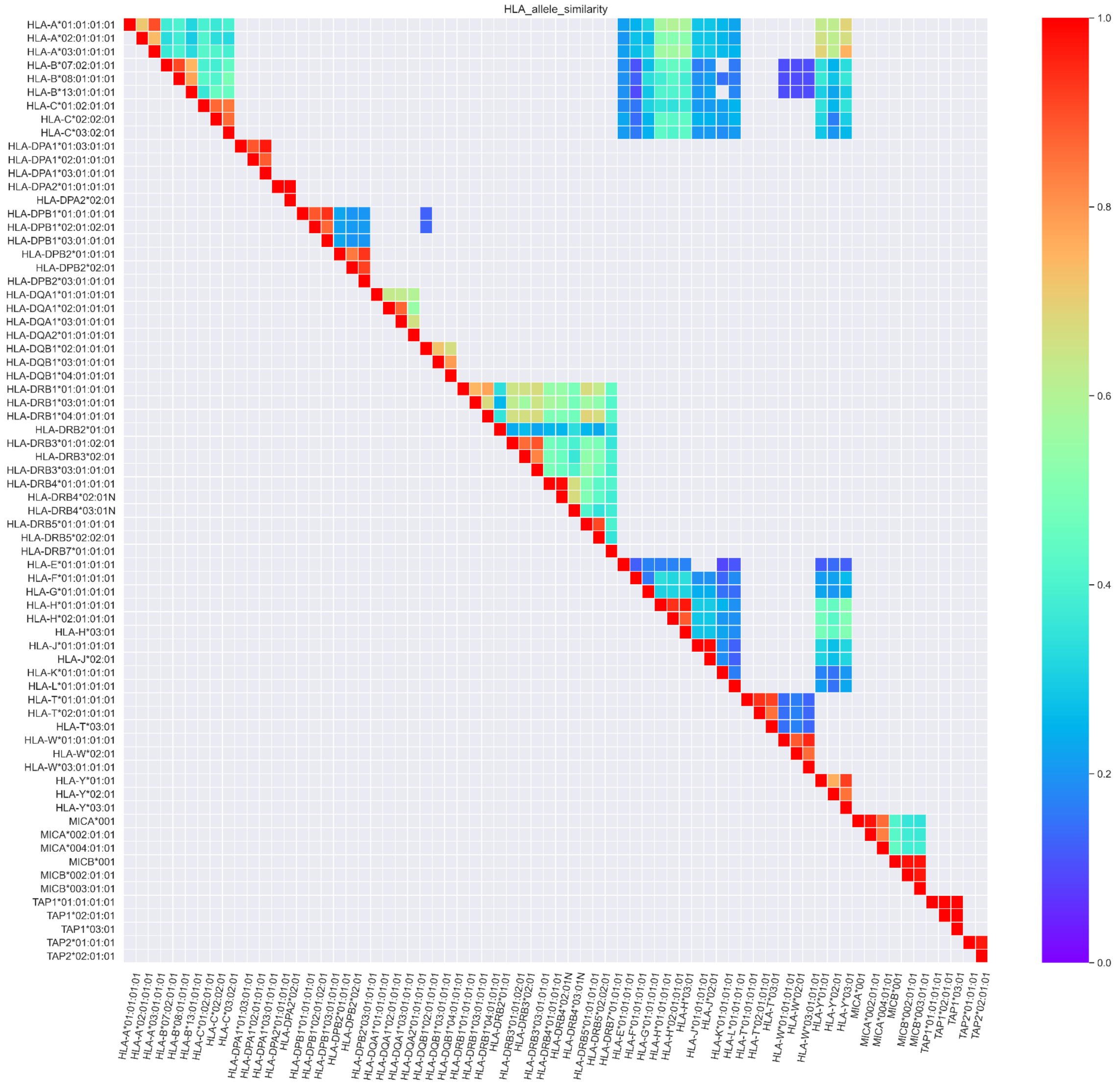


Figure S2. KIR allele calling accuracy when varying the dominant allele fraction threshold (--frac option in T1K) in the simulated data

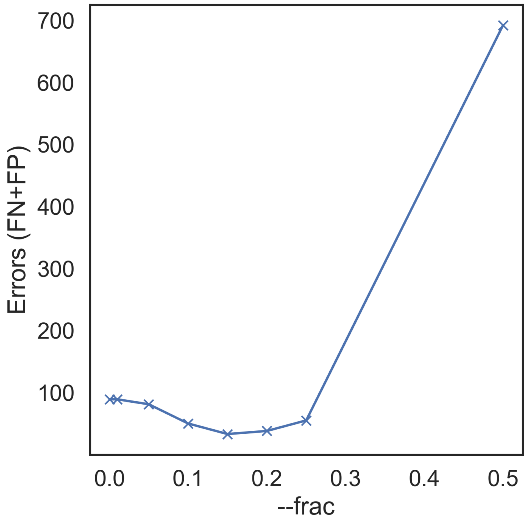


Figure S3. Evaluation of the unambiguous SNPs identified in T1K on the HPRC samples
The accuracy is 95.0% (57 validated SNPs over 60 total unambiguous SNPs).

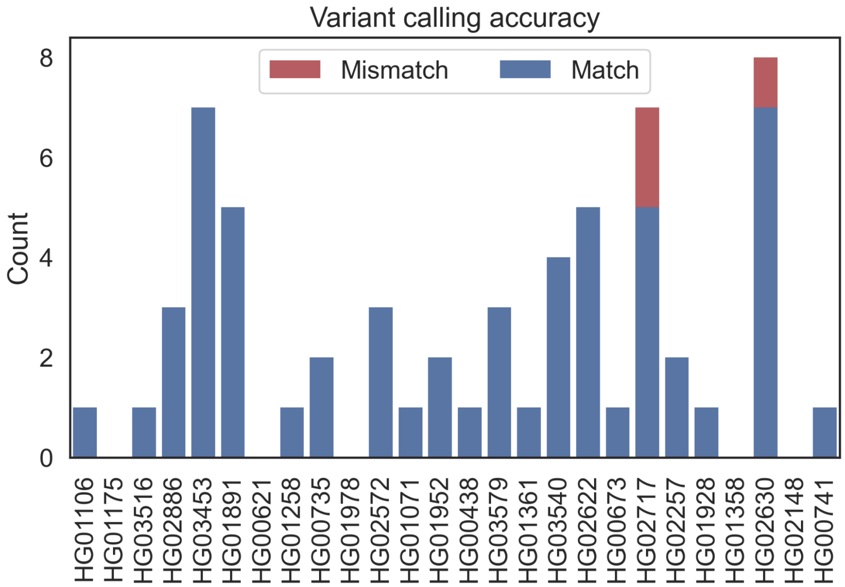
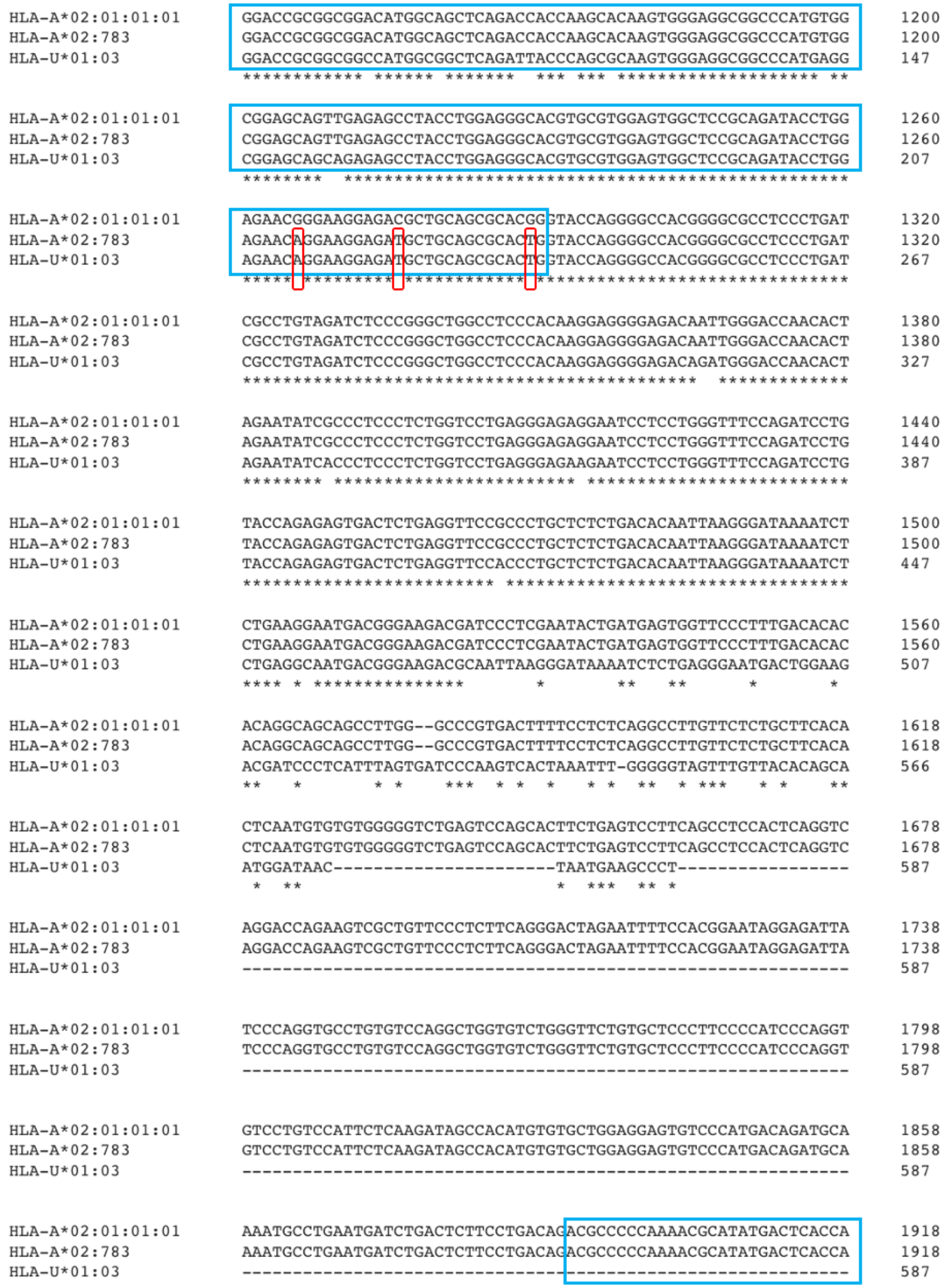
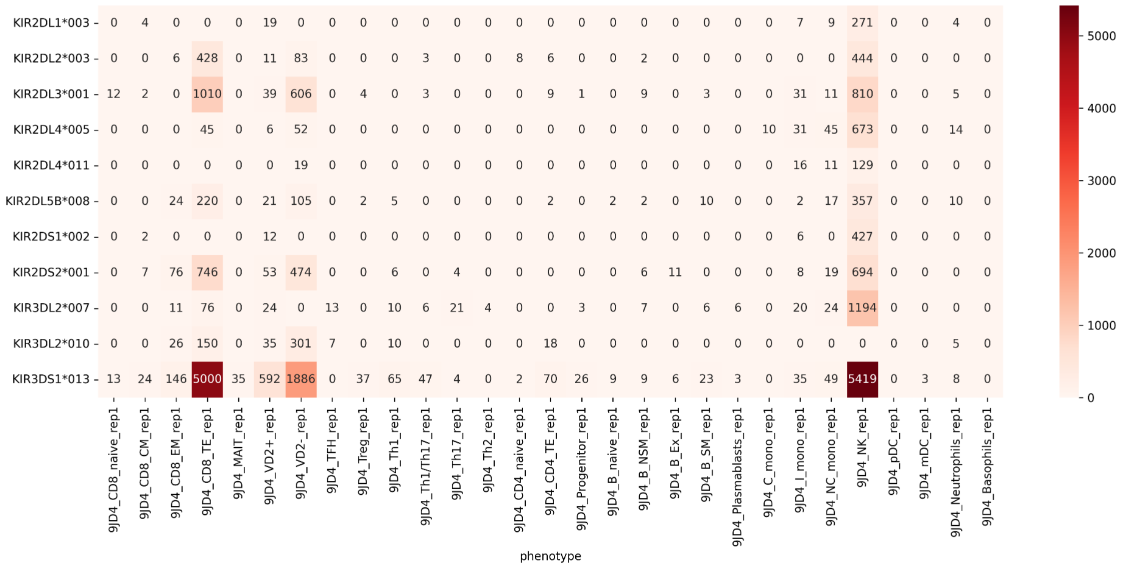


Figure S4. The multiple sequence alignments of HLA-A*02:01:01:01, HLA-A*02:783 and HLA-U*01:03's genomes, focusing on the exon 3 and 4 region of the HLA-A alleles. Blue rectangles mark the exon regions. Red rectangles mark the bases that are the same in HLA-A*02:783 and HLA-U*01:03, which create a 155bp exact match between these two alleles. This alignment was generated by https://www.ebi.ac.uk/Tools/msa/clustalo/ .

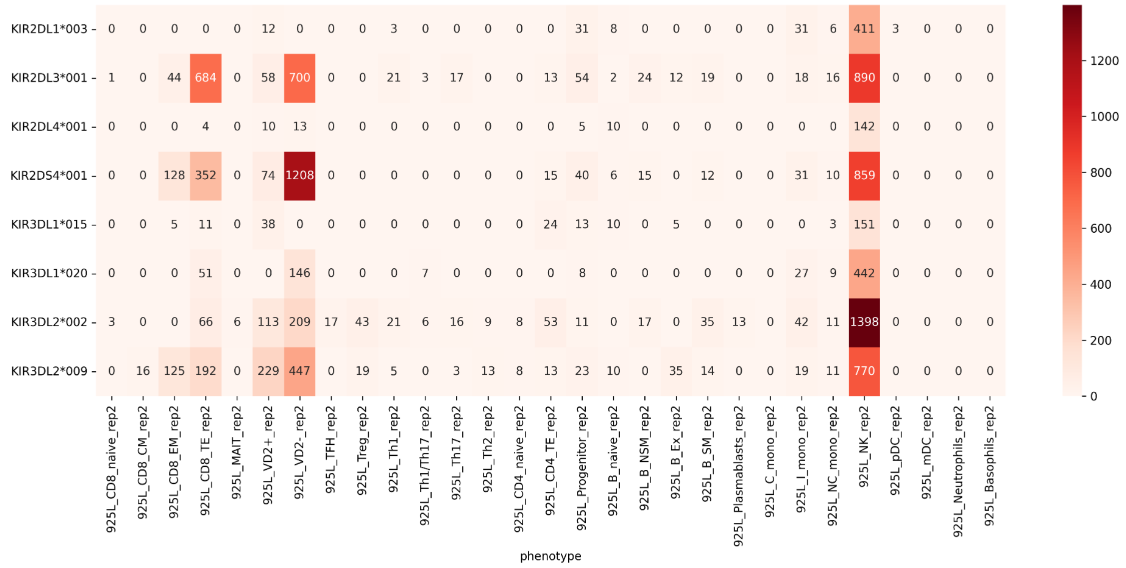


```
HLA-A*02:01:01:01      GGACCGCGGCGGACATGGCAGCTCAGACCACCAAGCACAAGTGGGAGGCGGCCCATGTGG      1200
HLA-A*02:783           GGACCGCGGCGGACATGGCAGCTCAGACCACCAAGCACAAGTGGGAGGCGGCCCATGTGG      1200
HLA-U*01:03            GGACCGCGGCGGCCATGGCGGCTCAGATTACCCAGCGCAAGTGGGAGGCGGCCCATGAGG      147
                       ************ ****** *******  *** *** ******************** **

HLA-A*02:01:01:01      CGGAGCAGTTGAGAGCCTACCTGGAGGGCACGTGCGTGGAGTGGCTCCGCAGATACCTGG      1260
HLA-A*02:783           CGGAGCAGTTGAGAGCCTACCTGGAGGGCACGTGCGTGGAGTGGCTCCGCAGATACCTGG      1260
HLA-U*01:03            CGGAGCAGCAGAGAGCCTACCTGGAGGGCACGTGCGTGGAGTGGCTCCGCAGATACCTGG      207
                       ********  **************************************************

HLA-A*02:01:01:01      AGAACGGGAAGGAGACGCTGCAGCGCACGGGTACCAGGGGCCACGGGGCGCCTCCCTGAT      1320
HLA-A*02:783           AGAACAGGAAGGAGATGCTGCAGCGCACTGGTACCAGGGGCCACGGGGCGCCTCCCTGAT      1320
HLA-U*01:03            AGAACAGGAAGGAGATGCTGCAGCGCACTGGTACCAGGGGCCACGGGGCGCCTCCCTGAT      267
                       ***** ********* ************ *******************************

HLA-A*02:01:01:01      CGCCTGTAGATCTCCCGGGCTGGCCTCCCACAAGGAGGGGAGACAATTGGGACCAACACT      1380
HLA-A*02:783           CGCCTGTAGATCTCCCGGGCTGGCCTCCCACAAGGAGGGGAGACAATTGGGACCAACACT      1380
HLA-U*01:03            CGCCTGTAGATCTCCCGGGCTGGCCTCCCACAAGGAGGGGAGACAGATGGGACCAACACT      327
                       *********************************************  *************

HLA-A*02:01:01:01      AGAATATCGCCCTCCCTCTGGTCCTGAGGGAGAGGAATCCTCCTGGGTTTCCAGATCCTG      1440
HLA-A*02:783           AGAATATCGCCCTCCCTCTGGTCCTGAGGGAGAGGAATCCTCCTGGGTTTCCAGATCCTG      1440
HLA-U*01:03            AGAATATCACCCTCCCTCTGGTCCTGAGGGAGAAGAATCCTCCTGGGTTTCCAGATCCTG      387
                       ******** ************************ **************************

HLA-A*02:01:01:01      TACCAGAGAGTGACTCTGAGGTTCCGCCCTGCTCTCTGACACAATTAAGGGATAAAATCT      1500
HLA-A*02:783           TACCAGAGAGTGACTCTGAGGTTCCGCCCTGCTCTCTGACACAATTAAGGGATAAAATCT      1500
HLA-U*01:03            TACCAGAGAGTGACTCTGAGGTTCCACCCTGCTCTCTGACACAATTAAGGGATAAAATCT      447
                       ************************* **********************************

HLA-A*02:01:01:01      CTGAAGGAATGACGGGAAGACGATCCCTCGAATACTGATGAGTGGTTCCCTTTGACACAC      1560
HLA-A*02:783           CTGAAGGAATGACGGGAAGACGATCCCTCGAATACTGATGAGTGGTTCCCTTTGACACAC      1560
HLA-U*01:03            CTGAGGCAATGACGGGAAGACGCAATTAAGGGATAAAATCTCTGAGGGAATGACTGGAAG      507
                       **** * ***************       *       **   **      *       *

HLA-A*02:01:01:01      ACAGGCAGCAGCCTTGG--GCCCGTGACTTTTCCTCTCAGGCCTTGTTCTCTGCTTCACA      1618
HLA-A*02:783           ACAGGCAGCAGCCTTGG--GCCCGTGACTTTTCCTCTCAGGCCTTGTTCTCTGCTTCACA      1618
HLA-U*01:03            ACGATCCCTCATTTAGTGATCCCAAGTCACTAAATTT-GGGGGTAGTTTGTTACACAGCA      566
                       **   *       * *    ***  * *  *   * *  **  * ***   * *    **

HLA-A*02:01:01:01      CTCAATGTGTGTGGGGGTCTGAGTCCAGCACTTCTGAGTCCTTCAGCCTCCACTCAGGTC      1678
HLA-A*02:783           CTCAATGTGTGTGGGGGTCTGAGTCCAGCACTTCTGAGTCCTTCAGCCTCCACTCAGGTC      1678
HLA-U*01:03            ATGGATAAC----------------------TAATGAAGCCCT-----------------      587
                        *  **                         *  ***  ** *

HLA-A*02:01:01:01      AGGACCAGAAGTCGCTGTTCCCTCTTCAGGGACTAGAATTTTCCACGGAATAGGAGATTA      1738
HLA-A*02:783           AGGACCAGAAGTCGCTGTTCCCTCTTCAGGGACTAGAATTTTCCACGGAATAGGAGATTA      1738
HLA-U*01:03            ------------------------------------------------------------      587

HLA-A*02:01:01:01      TCCCAGGTGCCTGTGTCCAGGCTGGTGTCTGGGTTCTGTGCTCCCTTCCCCATCCCAGGT      1798
HLA-A*02:783           TCCCAGGTGCCTGTGTCCAGGCTGGTGTCTGGGTTCTGTGCTCCCTTCCCCATCCCAGGT      1798
HLA-U*01:03            ------------------------------------------------------------      587

HLA-A*02:01:01:01      GTCCTGTCCATTCTCAAGATAGCCACATGTGTGCTGGAGGAGTGTCCCATGACAGATGCA      1858
HLA-A*02:783           GTCCTGTCCATTCTCAAGATAGCCACATGTGTGCTGGAGGAGTGTCCCATGACAGATGCA      1858
HLA-U*01:03            ------------------------------------------------------------      587

HLA-A*02:01:01:01      AAATGCCTGAATGATCTGACTCTTCCTGACAGACGCCCCCAAAACGCATATGACTCACCA      1918
HLA-A*02:783           AAATGCCTGAATGATCTGACTCTTCCTGACAGACGCCCCCAAAACGCATATGACTCACCA      1918
HLA-U*01:03            ------------------------------------------------------------      587
```

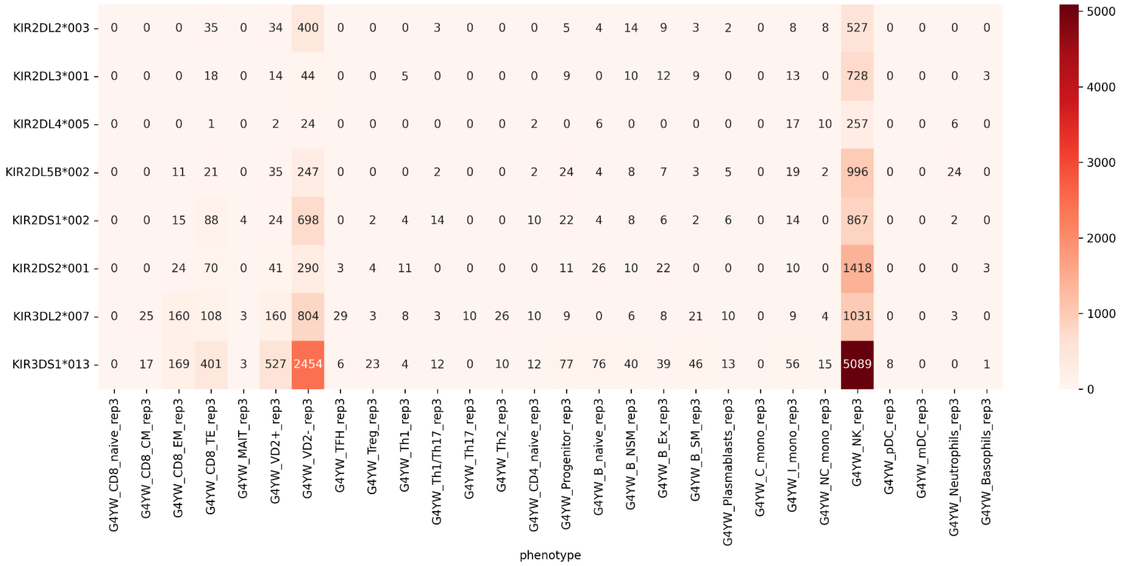
Figure S5. KIR allele expression (FPK) in Smart-seq scRNA-seq samples on 29 immune cells. Each heatmap corresponds to one donor.



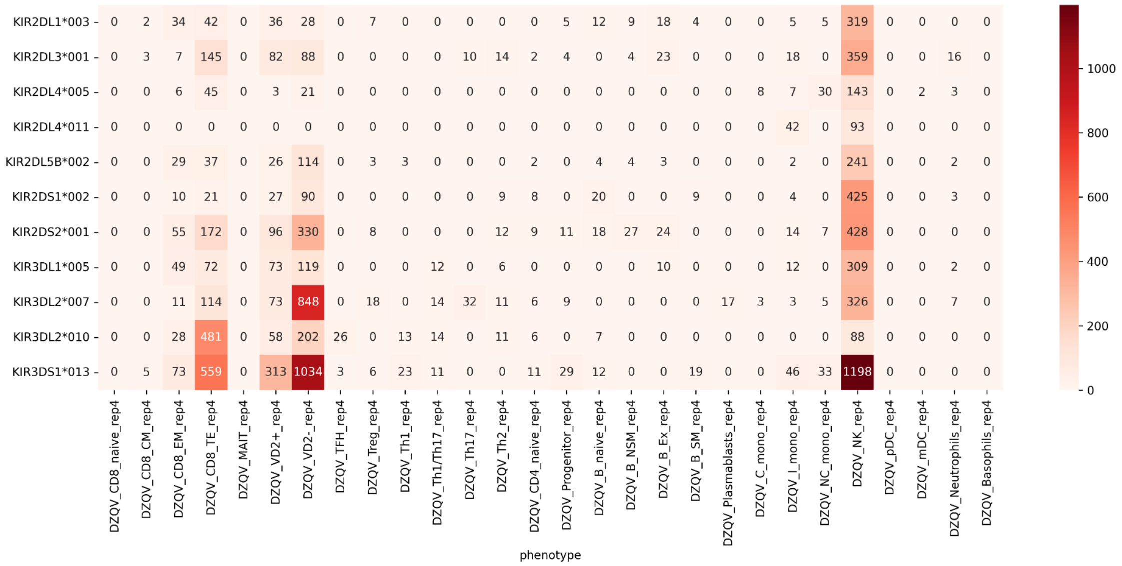
	9JD4_CD8_naive_rep1	9JD4_CD8_CM_rep1	9JD4_CD8_EM_rep1	9JD4_CD8_TE_rep1	9JD4_MAIT_rep1	9JD4_VD2+_rep1	9JD4_VD2-_rep1	9JD4_TFH_rep1	9JD4_Treg_rep1	9JD4_Th1_rep1	9JD4_Th1/Th17_rep1	9JD4_Th17_rep1	9JD4_Th2_rep1	9JD4_CD4_naive_rep1	9JD4_CD4_TE_rep1	9JD4_Progenitor_rep1	9JD4_B_naive_rep1	9JD4_B_NSM_rep1	9JD4_B_Ex_rep1	9JD4_B_SM_rep1	9JD4_Plasmablasts_rep1	9JD4_C_mono_rep1	9JD4_I_mono_rep1	9JD4_NC_mono_rep1	9JD4_NK_rep1	9JD4_pDC_rep1	9JD4_mDC_rep1	9JD4_Neutrophils_rep1	9JD4_Basophils_rep1
KIR2DL1*003	0	4	0	0	0	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	9	271	0	0	4	0
KIR2DL2*003	0	0	6	428	0	11	83	0	0	0	3	0	0	8	6	0	0	2	0	0	0	0	0	0	444	0	0	0	0
KIR2DL3*001	12	2	0	1010	0	39	606	0	4	0	3	0	0	0	9	1	0	9	0	3	0	0	31	11	810	0	0	5	0
KIR2DL4*005	0	0	0	45	0	6	52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	31	45	673	0	0	14	0
KIR2DL4*011	0	0	0	0	0	0	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	11	129	0	0	0	0
KIR2DL5B*008	0	0	24	220	0	21	105	0	2	5	0	0	0	0	2	0	2	2	0	10	0	0	2	17	357	0	0	10	0
KIR2DS1*002	0	2	0	0	0	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	427	0	0	0	0
KIR2DS2*001	0	7	76	746	0	53	474	0	0	6	0	4	0	0	0	0	0	6	11	0	0	0	8	19	694	0	0	0	0
KIR3DL2*007	0	0	11	76	0	24	0	13	0	10	6	21	4	0	0	3	0	7	0	6	6	0	20	24	1194	0	0	0	0
KIR3DL2*010	0	0	26	150	0	35	301	7	0	10	0	0	0	0	18	0	0	0	0	0	0	0	0	0	0	0	0	5	0
KIR3DS1*013	13	24	146	5000	35	592	1886	0	37	65	47	4	0	2	70	26	9	9	6	23	3	0	35	49	5419	0	3	8	0



	925L_CD8_naive_rep2	925L_CD8_CM_rep2	925L_CD8_EM_rep2	925L_CD8_TE_rep2	925L_MAIT_rep2	925L_VD2+_rep2	925L_VD2-_rep2	925L_TFH_rep2	925L_Treg_rep2	925L_Th1_rep2	925L_Th1/Th17_rep2	925L_Th17_rep2	925L_Th2_rep2	925L_CD4_naive_rep2	925L_CD4_TE_rep2	925L_Progenitor_rep2	925L_B_naive_rep2	925L_B_NSM_rep2	925L_B_Ex_rep2	925L_B_SM_rep2	925L_Plasmablasts_rep2	925L_C_mono_rep2	925L_I_mono_rep2	925L_NC_mono_rep2	925L_NK_rep2	925L_pDC_rep2	925L_mDC_rep2	925L_Neutrophils_rep2	925L_Basophils_rep2
KIR2DL1*003	0	0	0	0	0	12	0	0	0	3	0	0	0	0	0	31	8	0	0	0	0	0	31	6	411	3	0	0	0
KIR2DL3*001	1	0	44	684	0	58	700	0	0	21	3	17	0	0	13	54	2	24	12	19	0	0	18	16	890	0	0	0	0
KIR2DL4*001	0	0	0	4	0	10	13	0	0	0	0	0	0	0	0	5	10	0	0	0	0	0	0	0	142	0	0	0	0
KIR2DS4*001	0	0	128	352	0	74	1208	0	0	0	0	0	0	0	15	40	6	15	0	12	0	0	31	10	859	0	0	0	0
KIR3DL1*015	0	0	5	11	0	38	0	0	0	0	0	0	0	0	24	13	10	0	5	0	0	0	0	3	151	0	0	0	0
KIR3DL1*020	0	0	0	51	0	0	146	0	0	0	7	0	0	0	0	8	0	0	0	0	0	0	27	9	442	0	0	0	0
KIR3DL2*002	3	0	0	66	6	113	209	17	43	21	6	16	9	8	53	11	0	17	0	35	13	0	42	11	1398	0	0	0	0
KIR3DL2*009	0	16	125	192	0	229	447	0	19	5	0	3	13	8	13	23	10	0	35	14	0	0	19	11	770	0	0	0	0

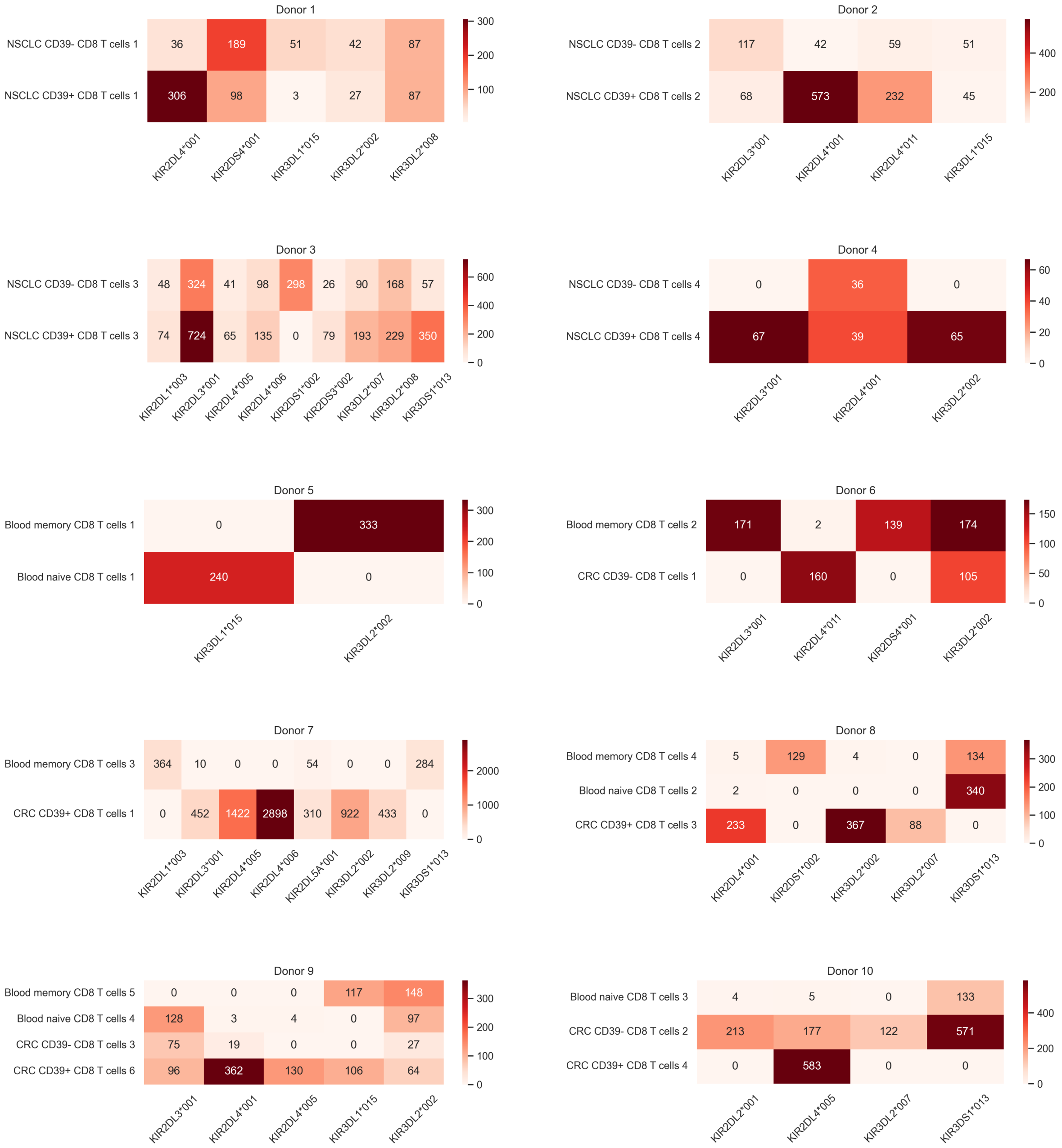


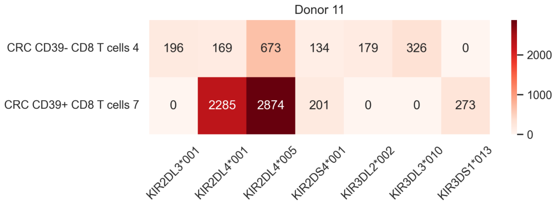
	G4YW_CD8_naive_rep3	G4YW_CD8_CM_rep3	G4YW_CD8_EM_rep3	G4YW_CD8_TE_rep3	G4YW_MAIT_rep3	G4YW_VD2+_rep3	G4YW_VD2-_rep3	G4YW_TFH_rep3	G4YW_Treg_rep3	G4YW_Th1_rep3	G4YW_Th1/Th17_rep3	G4YW_Th17_rep3	G4YW_Th2_rep3	G4YW_CD4_naive_rep3	G4YW_Progenitor_rep3	G4YW_B_naive_rep3	G4YW_B_NSM_rep3	G4YW_B_Ex_rep3	G4YW_B_SM_rep3	G4YW_Plasmablasts_rep3	G4YW_C_mono_rep3	G4YW_I_mono_rep3	G4YW_NC_mono_rep3	G4YW_NK_rep3	G4YW_pDC_rep3	G4YW_mDC_rep3	G4YW_Neutrophils_rep3	G4YW_Basophils_rep3
KIR2DL2*003	0	0	0	35	0	34	400	0	0	0	3	0	0	0	5	4	14	9	3	2	0	8	8	527	0	0	0	0
KIR2DL3*001	0	0	0	18	0	14	44	0	0	5	0	0	0	0	9	0	10	12	9	0	0	13	0	728	0	0	0	3
KIR2DL4*005	0	0	0	1	0	2	24	0	0	0	0	0	0	2	0	6	0	0	0	0	0	17	10	257	0	0	6	0
KIR2DL5B*002	0	0	11	21	0	35	247	0	0	0	2	0	0	2	24	4	8	7	3	5	0	19	2	996	0	0	24	0
KIR2DS1*002	0	0	15	88	4	24	698	0	2	4	14	0	0	10	22	4	8	6	2	6	0	14	0	867	0	0	2	0
KIR2DS2*001	0	0	24	70	0	41	290	3	4	11	0	0	0	0	11	26	10	22	0	0	0	10	0	1418	0	0	0	3
KIR3DL2*007	0	25	160	108	3	160	804	29	3	8	3	10	26	10	9	0	6	8	21	10	0	9	4	1031	0	0	3	0
KIR3DS1*013	0	17	169	401	3	527	2454	6	23	4	12	0	10	12	77	76	40	39	46	13	0	56	15	5089	8	0	0	1

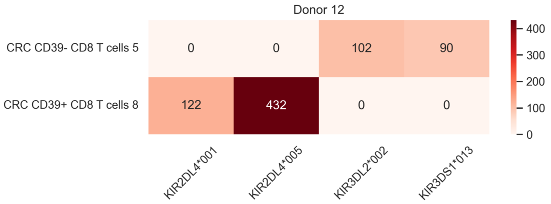


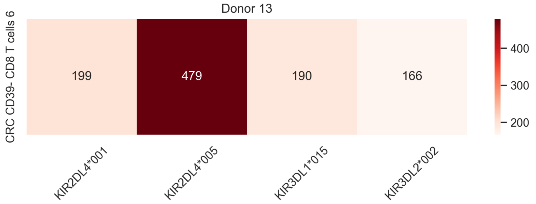
	DZQV_CD8_naive_rep4	DZQV_CD8_CM_rep4	DZQV_CD8_EM_rep4	DZQV_CD8_TE_rep4	DZQV_MAIT_rep4	DZQV_VD2+_rep4	DZQV_VD2-_rep4	DZQV_TFH_rep4	DZQV_Treg_rep4	DZQV_Th1_rep4	DZQV_Th1/Th17_rep4	DZQV_Th17_rep4	DZQV_Th2_rep4	DZQV_CD4_naive_rep4	DZQV_Progenitor_rep4	DZQV_B_naive_rep4	DZQV_B_NSM_rep4	DZQV_B_Ex_rep4	DZQV_B_SM_rep4	DZQV_Plasmablasts_rep4	DZQV_C_mono_rep4	DZQV_I_mono_rep4	DZQV_NC_mono_rep4	DZQV_NK_rep4	DZQV_pDC_rep4	DZQV_mDC_rep4	DZQV_Neutrophils_rep4	DZQV_Basophils_rep4
KIR2DL1*003	0	2	34	42	0	36	28	0	7	0	0	0	0	0	5	12	9	18	4	0	0	5	5	319	0	0	0	0
KIR2DL3*001	0	3	7	145	0	82	88	0	0	0	0	10	14	2	4	0	4	23	0	0	0	18	0	359	0	0	16	0
KIR2DL4*005	0	0	6	45	0	3	21	0	0	0	0	0	0	0	0	0	0	0	0	0	8	7	30	143	0	2	3	0
KIR2DL4*011	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	42	0	93	0	0	0	0
KIR2DL5B*002	0	0	29	37	0	26	114	0	3	3	0	0	0	2	0	4	4	3	0	0	0	2	0	241	0	0	2	0
KIR2DS1*002	0	0	10	21	0	27	90	0	0	0	0	0	9	8	0	20	0	0	9	0	0	4	0	425	0	0	3	0
KIR2DS2*001	0	0	55	172	0	96	330	0	8	0	0	0	12	9	11	18	27	24	0	0	0	14	7	428	0	0	0	0
KIR3DL1*005	0	0	49	72	0	73	119	0	0	0	12	0	6	0	0	0	0	10	0	0	0	12	0	309	0	0	2	0
KIR3DL2*007	0	0	11	114	0	73	848	0	18	0	14	32	11	6	9	0	0	0	0	17	3	3	5	326	0	0	7	0
KIR3DL2*010	0	0	28	481	0	58	202	26	0	13	14	0	11	6	0	7	0	0	0	0	0	0	0	88	0	0	0	0
KIR3DS1*013	0	5	73	559	0	313	1034	3	6	23	11	0	0	11	29	12	0	0	19	0	0	46	33	1198	0	0	0	0

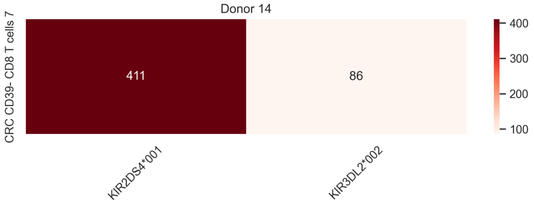
Figure S6. KIR allele expression (FPK) in Smart-seq scRNA-seq samples on CD8+ T cells. Each heatmap corresponds to the cells bearing the same set of HLA class I genes. Donor 8 is the example in Figure 2A.

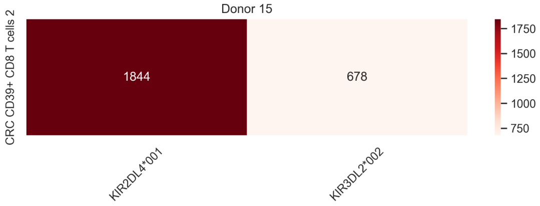



Donor 11
CRC CD39- CD8 T cells 4
CRC CD39+ CD8 T cells 7
196
169
673
134
179
326
0
0
2285
2874
201
0
0
273
KIR2DL3*001
KIR2DL4*001
KIR2DL4*005
KIR2DS4*001
KIR3DL2*002
KIR3DL3*010
KIR3DS1*013
2000
1000
0


Donor 12
CRC CD39- CD8 T cells 5
CRC CD39+ CD8 T cells 8
0
0
102
90
122
432
0
0
KIR2DL4*001
KIR2DL4*005
KIR3DL2*002
KIR3DS1*013
400
300
200
100
0


Donor 13
CRC CD39- CD8 T cells 6
199
479
190
166
KIR2DL4*001
KIR2DL4*005
KIR3DL1*015
KIR3DL2*002
400
300
200


Donor 14
CRC CD39- CD8 T cells 7
411
86
KIR2DS4*001
KIR3DL2*002
400
300
200
100


Donor 15
CRC CD39+ CD8 T cells 2
1844
678
KIR2DL4*001
KIR3DL2*002
1750
1500
1250
1000
750

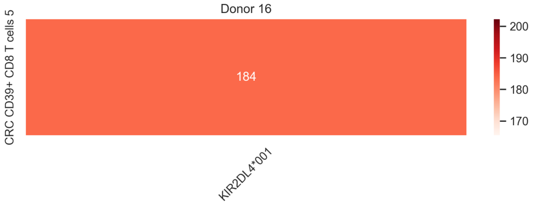

Donor 16
CRC CD39+ CD8 T cells 5
184
KIR2DL4*001
200
190
180
170

Figure S7. Illustration of the novel SNP identification procedure
Green and yellow boxes represent the allele-specific variations. Red dots represent mismatches on the read alignments. The third combination with the orange star is the final SNP result that can improve all five alignments with only one introduced SNP.