Table 1a. Details of healthy donors

Donor No.	Gender	Age Range (Years)*	HLA-DR type
Healthy 01	Male	21-30	DRB1*03(DR17), DRB1*04
Healthy 02	Female	21-30	DRB1*04, DRB1*13
Healthy 03	Female	31-40	DRB1*04, DRB1*14
Healthy 04	Female	31-40	DRB1*04, DRB1*14
Healthy 05	Male	31-40	DRB1*04, DRB1*08
Healthy 06	Male	41-50	DRB1*04, DRB1*04
Healthy 07	Female	31-40	DRB1*04, DRB1*04
Healthy 08	Male	41-50	DRB1*04, DRB1*07
Healthy 09	Female	31-40	DRB1*03(DR17), DRB1*04
Healthy 11	Male	31-40	DRB1*04, DRB1*15
Healthy 12	Male	41-50	DRB1*0103, DRB1*13
Healthy 13	Male	21-30	DRB1*0103, DRB1*15
Healthy 14	Male	51-60	DRB1*01, DRB1*09
Healthy 15	Male	41-50	DRB1*01, DRB1*12
Healthy 16	Female	31-40	DRB1*01, DRB1*15
Healthy 17	Female	51-60	DRB1*03(DR17), DRB1*07
Healthy 18	Female	41-50	DRB1*03(DR17), DRB1*07
Healthy 19	Male	31-40	DRB1*03(DR17), DRB1*03

*Subjects donated blood samples > once, therefore age range given.

Table 1b. Details of colorectal cancer patients.

Donor No.	Gender	Age (Years)	HLA-DR type	Stage	Treatment	Histology diagnosis	Time of sample collection
CRC01	Female	73	DRB1*04 DRB1*15	T3N0M0 Stage III	Surgery + 6 months adjuvant chemotherapy	adenocarcinoma	7 years after surgery
CRC02	Male	80	DRB1*04 DRB1*15	T3N2M0 Stage III	Surgery + 6 months adjuvant chemotherapy	adenocarcinoma	8 years after surgery
CRC03	Male	72	DRB1*04 DRB1*07	T3N1M0 Stage III	Surgery + 3 months adjuvant chemotherapy*	adenocarcinoma	9 years after surgery
CRC04	Male	69	DRB1*01 DRB1*04	T2N0M0 Stage II	Surgery	adenocarcinoma	During surgery
CRC05	Male	83	DRB1*01 DRB1*04	T3N0M0 Stage III	Surgery	adenocarcinoma	During surgery
CRC06	Male	63	DRB1*04 DRB1*15	T2N0M0Stage II	Surgery	Square cell carcinoma	During surgery
CRC07	Male	69	DRB1*03 DRB1*04	T3N0M0Stage III	Surgery	adenocarcinoma	During surgery
CRC08	Male	72	DRB1*01 DRB1*04	T3N1M0Stage III	Surgery	adenocarcinoma	During surgery
CRC09	Male	72	DRB1*03 DRB1*04	T2N0M0Stage II	Surgery	adenocarcinoma	During surgery

CRC10	Male	76	DRB1*04 DRB1*04	T3N0M0S tage III	Surgery	adenocarcinoma	During surgery
CRC11	Male	66	DRB1*03 DRB1*04	T2N0M0S tage II	Surgery	adenocarcinoma	During surgery
CRC12	Male	62	DRB1*04 DRB1*11	T3N1M0S tage III	Surgery	adenocarcinoma	During surgery

*Standard 6 months chemotherapy did not finish due to intolerance.

Table 2a Sequence of overlapping DNAJB7 peptides

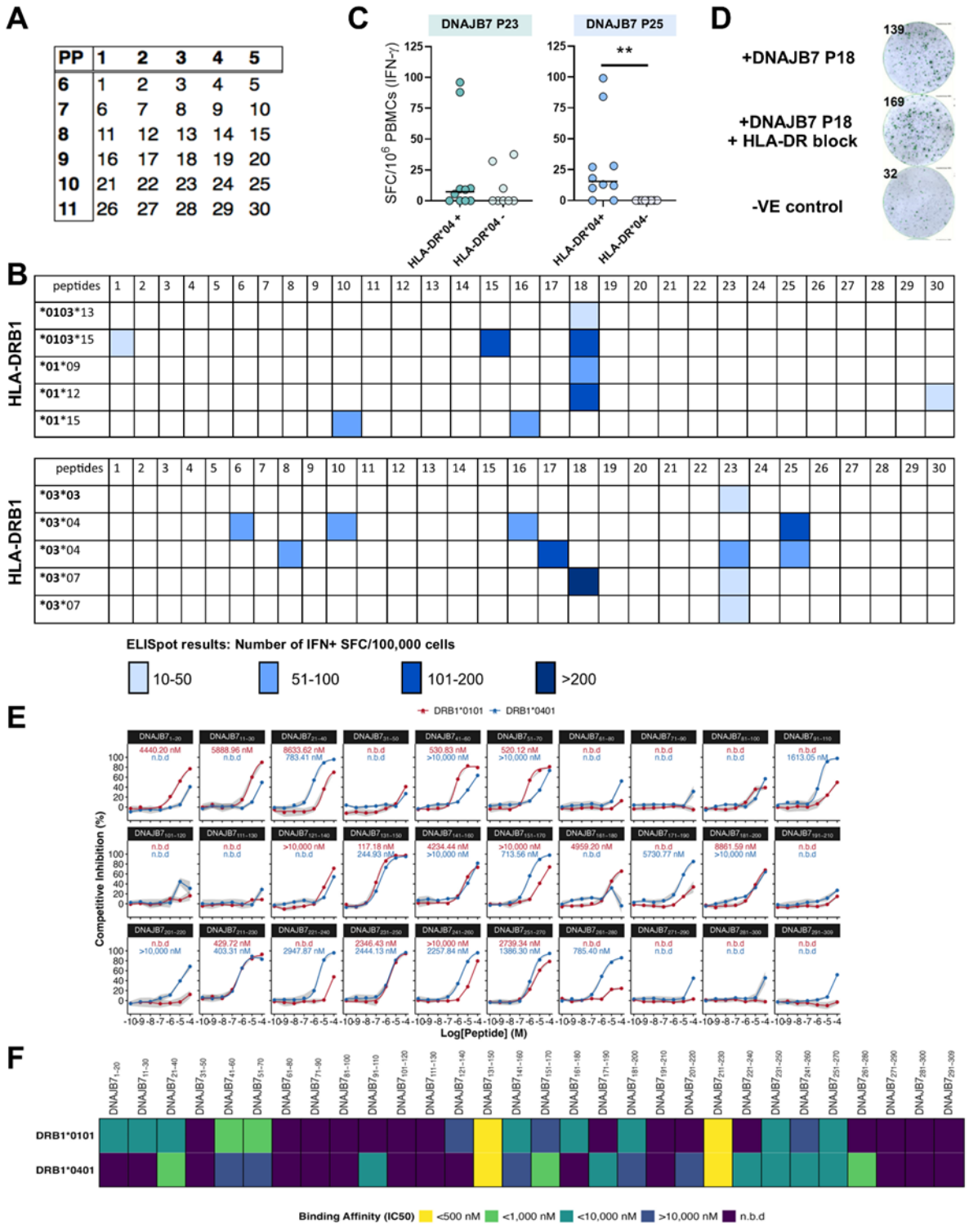
peptide label	DNAJB7 overlapping peptide sequence
1	MVDYYEVLGLQRYASPEDIK
2	QRYASPEDIKKAYHKVALKW
3	KAYHKVALKWHPDKNPENKE
4	HPDKNPENKEEAERKFKEVA
5	EAERKFKEVAEAYEVLSNDE
6	EAYEVLSNDEKRDIYDKYGT
7	KRDIYDKYGTEGLNGGGSHF
8	EGLNGGGSHFDDECEYGFTF
9	DDECEYGFTFHKPDDVFKEI
10	HKPDDVFKEIFHERDPFSFH
11	FHERDPFSFHFFEDSLEDLL
12	FFEDSLEDLLNRPGSSYGNR
13	NRPGSSYGNRNRDAGYFFST
14	NRDAGYFFSTASEYPIFEKF
15	ASEYPIFEKFSSYDTGYTSQ
16	SSYDTGYTSQGSLGHEGLTS
17	GSLGHEGLTSFSSLAFDNSG
18	FSSLAFDNSGMDNYISVTTS
19	MDNYISVTTSDKIVNGRNIN
20	DKIVNGRNINTKKIIESDQE
21	TKKIIESDQEREAEDNGELT
22	REAEDNGELTFFLVNSVANE
23	FFLVNSVANEEGFAKECSWR
24	EGFAKECSWRTQSFNNYSPN
25	TQSFNNYSPNSHSSKHVSQY
26	SHSSKHVSQYTFVDNDEGGI
27	TFVDNDEGGISWVTSNRDPP
28	SWVTSNRDPPIFSAGVKEGG
29	IFSAGVKEGGKRKKKKRKEV
30	KRKKKKRKEVQKKSTKRNC

Table 2b Sequence of truncated peptides of P23 and P25

P23 truncated peptide sequence	P25 truncated peptide sequence
FFLVNSVANEEGFAKECSWR	TQSFNNYSPNSHSSKHVSQY
FFLVNSVANEEGFAKECSW	TQSFNNYSPNSHSSKHVSQ
FFLVNSVANEEGFAKECS	TQSFNNYSPNSHSSKHVS
FFLVNSVANEEGFAKEC	TQSFNNYSPNSHSSKHV
FFLVNSVANEEGFAKE	TQSFNNYSPNSHSSKH
FFLVNSVANEEGFAK	TQSFNNYSPNSHSSK
FFLVNSVANEEGFA	TQSFNNYSPNSHSS
FFLVNSVANEEGF	TQSFNNYSPNSHS
FFLVNSVANEEG	TQSFNNYSPNSH
FFLVNSVANEE	TQSFNNYSPNS
FLVNSVANEEGFAKECSWR	QSFNNYSPNSHSSKHVSQY
LVNSVANEEGFAKECSWR	SFNNYSPNSHSSKHVSQY
VNSVANEEGFAKECSWR	FNNYSPNSHSSKHVSQY
NSVANEEGFAKECSWR	NNYSPNSHSSKHVSQY
SVANEEGFAKECSWR	NYSPNSHSSKHVSQY
VANEEGFAKECSWR	YSPNSHSSKHVSQY
ANEEGFAKECSWR	SPNSHSSKHVSQY
NEEGFAKECSWR	PNSHSSKHVSQY
EEGFAKECSWR	NSHSSKHVSQY

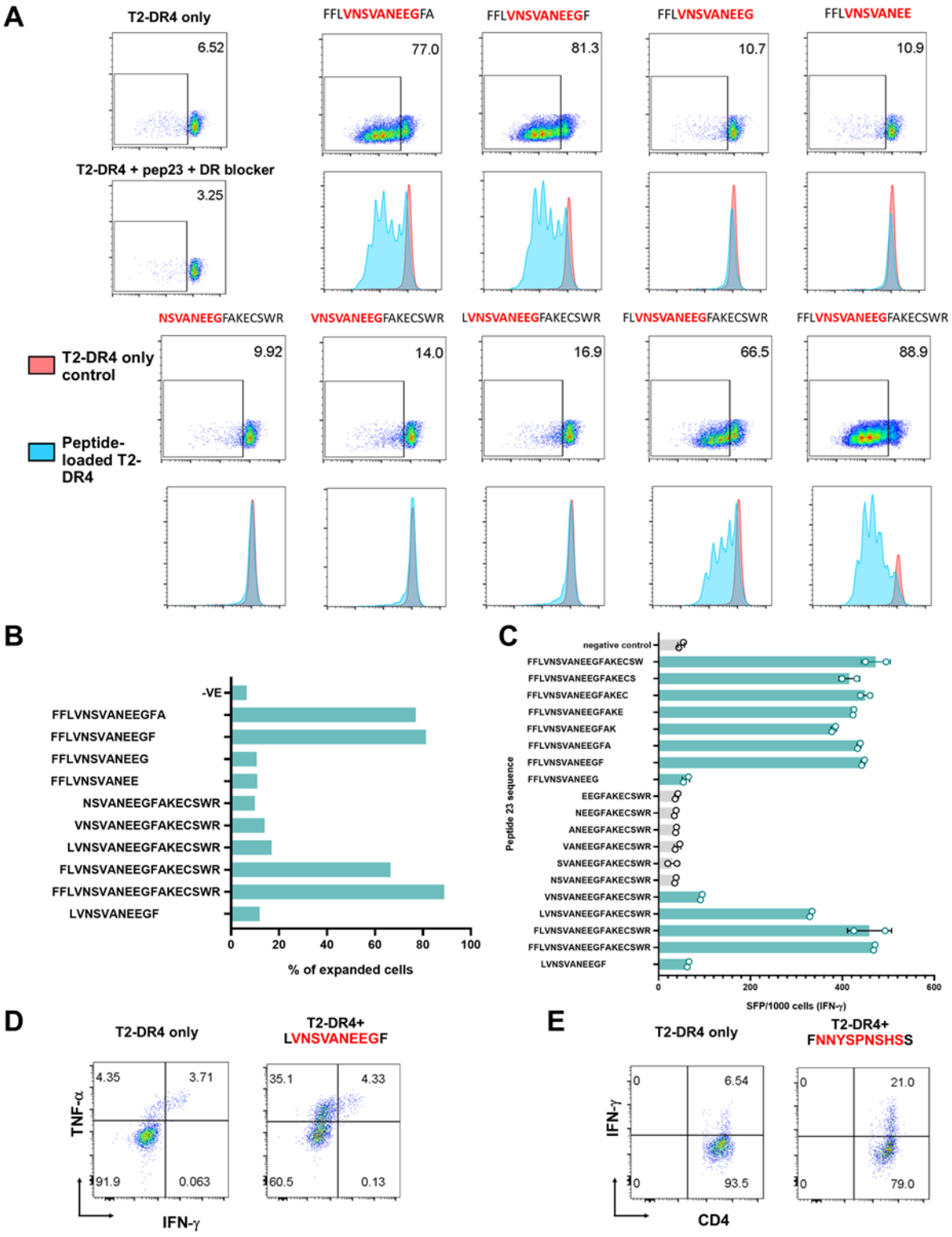
Table 2c Sequence of flank modified peptides of P23 and P25

P23 flank modified peptides		P25 flank modified peptides	
peptide labels	peptide sequence	peptide labels	peptide sequence
-2Y	F**Y**LVNSVANEEGFAK	-2Y	TQ**Y**FNNYSPNSHSSKH
-2E	F**E**LVNSVANEEGFAK	-2E	TQ**E**FNNYSPNSHSSKH
-2L	F**L**LVNSVANEEGFAK	-2L	TQ**L**FNNYSPNSHSSKH
-2S	F**S**LVNSVANEEGFAK	-2T	TQ**T**FNNYSPNSHSSKH
-2R	F**R**LVNSVANEEGFAK	-2R	TQ**R**FNNYSPNSHSSKH
11Y	FFLVNSVANEEGF**Y**K	11Y	TQSFNNYSPNSHSS**Y**H
11E	FFLVNSVANEEGF**E**K	11E	TQSFNNYSPNSHSS**E**H
11L	FFLVNSVANEEGF**L**K	11L	TQSFNNYSPNSHSS**L**H
11S	FFLVNSVANEEGF**S**K	11S	TQSFNNYSPNSHSS**S**H
11R	FFLVNSVANEEGF**R**K	11R	TQSFNNYSPNSHSS**R**H



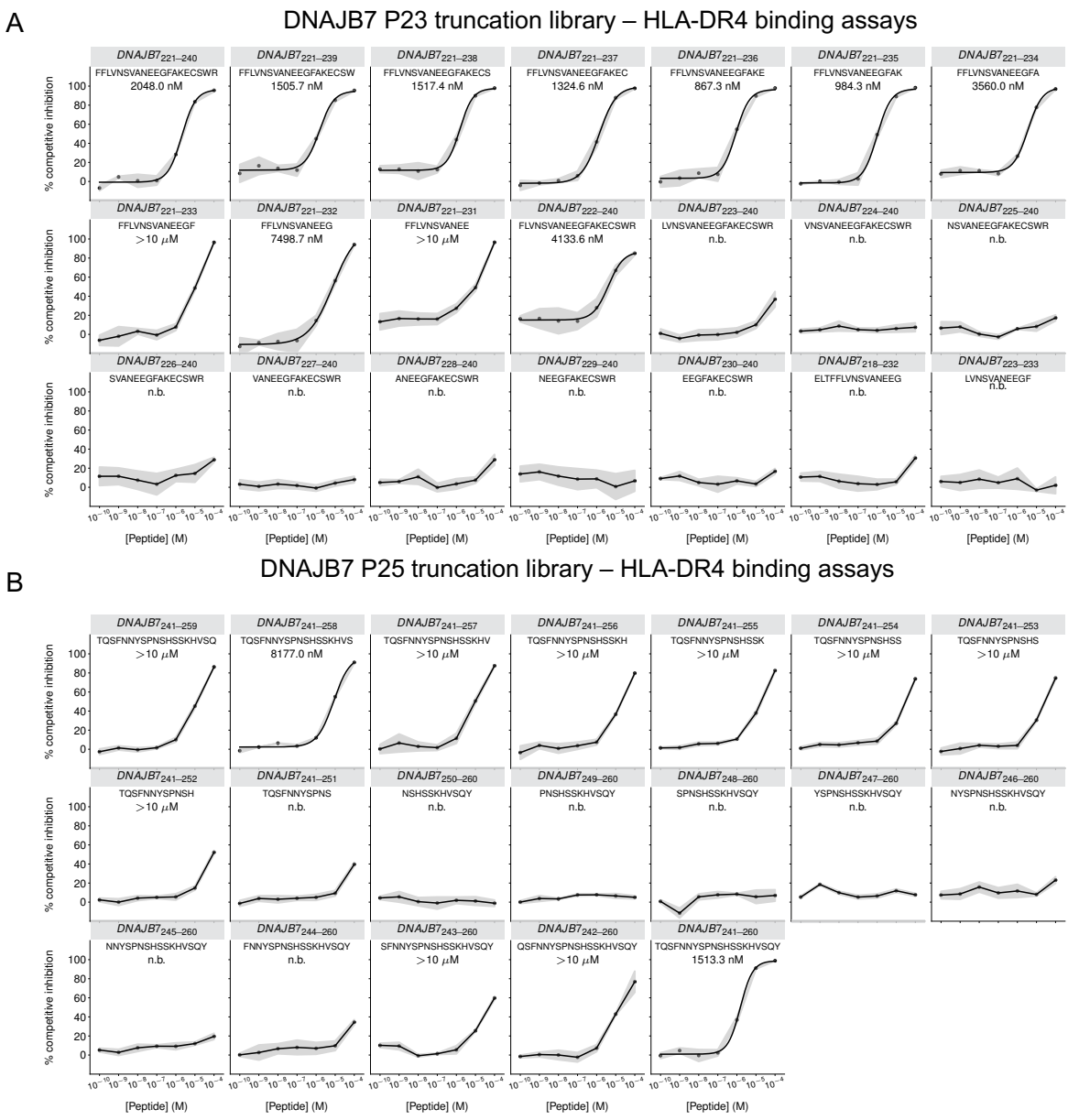
Supplementary Figure 1. Mapping DNAJB7 CD4 T cell epitopes in HLA-DR1, DR3 and DR4 donors (A) 30 overlapping DNAJB7 peptides (listed in Supplementary Table 2) were aliquoted into 11 peptide pools (B) Screening DNAJB7 T cell responses to peptides in HLA-DR1+ and DR3+ donors. (C) Summary of IFN-γ ELISpot results of HLA-DR4+ donors (N=10) versus HLA-

DR4- donors (N=8) who responded to either P23 or P25, respectively. (D) Representative IFN-γ ELISpot of T cells form HLA-DR1+ donors responded to DNAJB7 P18, with or without anti-HLA-DR blocking. (E) Competitive inhibition curves of DNAJB7 overlapping peptide library binding to HLA-DR1 (red) and HLA-DR4 (blue). Data representative of 2-3 independent experiments, each with 3 technical replicates. Representative replicate is shown, presented as mean percentage inhibition (circles) with standard deviation (shaded area). An IC_{50} value estimating binding affinity (inset) is shown where fitting resulted in values of <10 μM (curve fit, black line). Peptides with poor curve fit and weak binding (>10 μM) or no binding detected (n.b.d.) have mean values that are connected via straight lines. (F) Summative heatmap of HLA-DR1 and HLA-DR4 binding IC50s from experiments described in figure E.

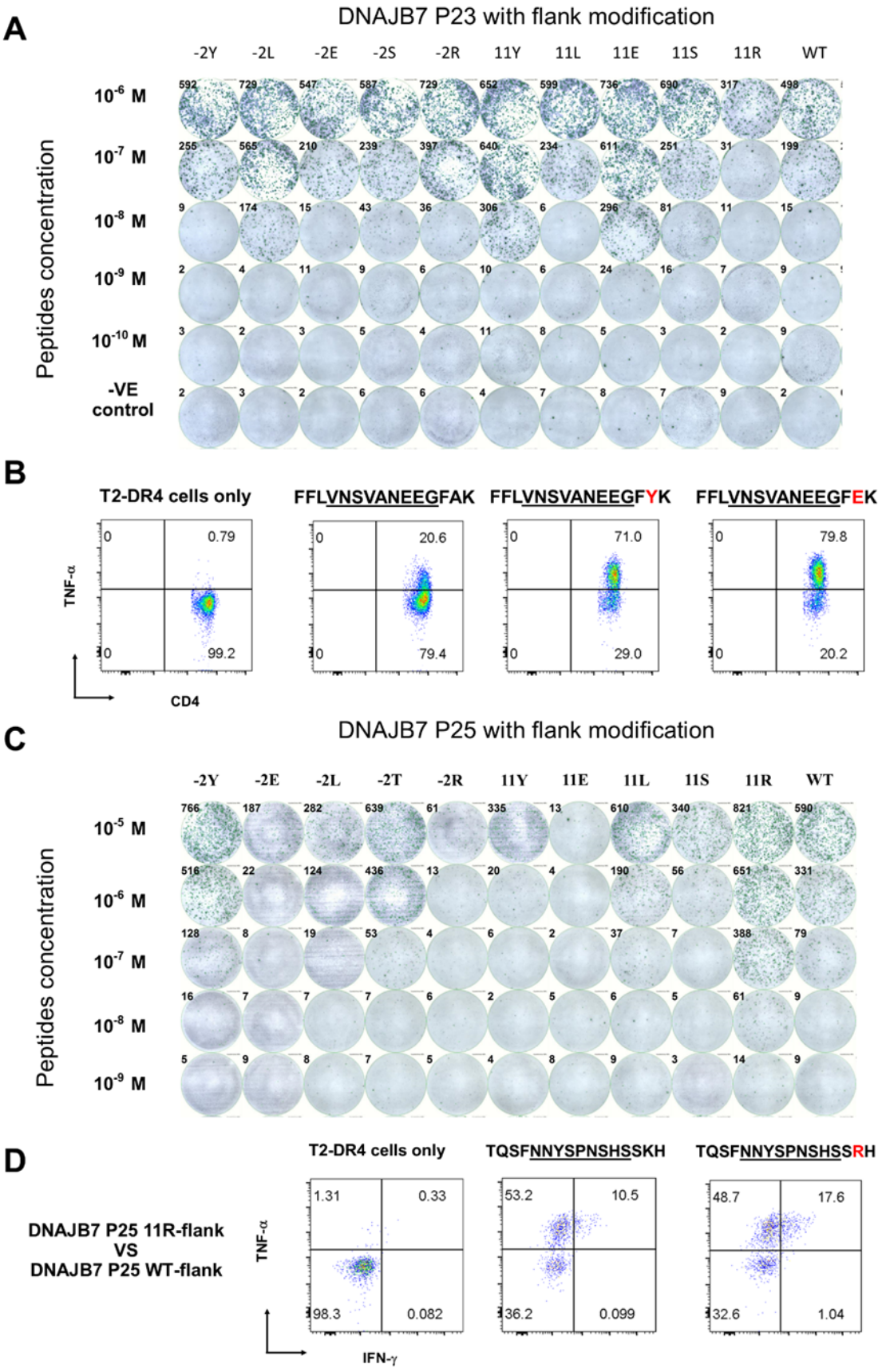


Supplementary Figure 2. Analysis of epitope binding core of DNAJB7 P23 (DNAJB7$_{221-240}$) using functional assays. (A) DNAJB7 221-240 T cell line was labelled with Cell Trace Violet, and co-cultured with T2-DR4 cells (ratio 1:1) loaded with P23 (DNAJB7221-240) variants, comprising serial truncation from the N- and C-termini. T cell lines co-cultured with empty T2-DR4 cells,

and T2-DR4 cells pulsed with P23 (DNAJB7$_{221-240}$), plus anti-HLA-DR blocking antibody were used as control. Dilution of trace violet was measured by flow cytometry on day 5. P23 (DNAJB7$_{221-240}$) truncation analysis on P23 (DNAJB7$_{221-240}$) specific $CD4^+$ T cell line from HC02. (B) P23-specific T cell lines were stimulated with peptide variants and proliferation measured. (C) Bar chart representing the percentage of ELISpot results of IFN-γ producing $CD4^+$ T cell cultured with P23 (DNAJB7$_{221-240}$) variant-pulsed T2-DR4 cells in a $CD4^+$ T cell line. (D) T cell line producing TNF-α and IFN-γ was assessed by ICS in response to overnight co-culture with T2-DR4 cells and peptide L<u>VNSVANEEG</u>F. (E) T cell line producing IFN-γ was assessed by ICS in response to overnight co-culture with T2-DR4 cells and peptide F<u>NNYSPNSHS</u>S.

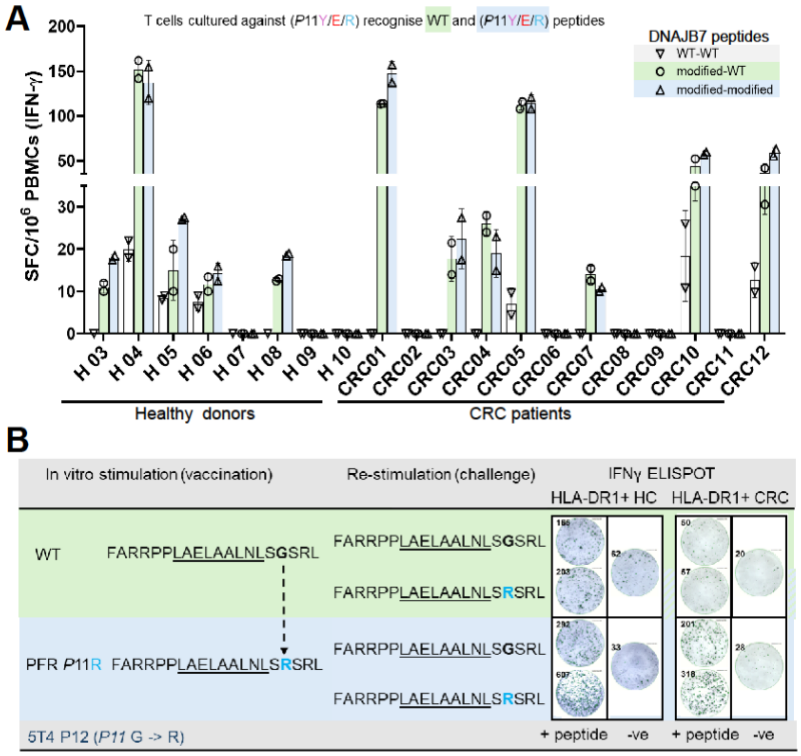


Supplementary Figure 3. Peptide binding of truncated DNAJB7 P23 and P25 peptides to HLA-DR4 via competitive inhibition assays. A) Competitive inhibition curves of DNAJB7 P23 truncated peptides binding to HLA-DR4. Data representative of n = 2-3 independent experiments, each with n = 3 technical replicates. Representative replicate is shown, presented as mean percentage inhibition (circles) with standard deviation (shaded area). An IC50 value defining binding (inset) is shown where fitting resulted in values of <10 μM (curve fit, black line). Peptides with poor curve fit and weak binding (>10 μM) or no binding (n.b.) have mean values that are connected via straight lines. B) Competitive inhibition curves of DNAJB7 P25 truncated peptides binding to HLA-DR4 as described in A).

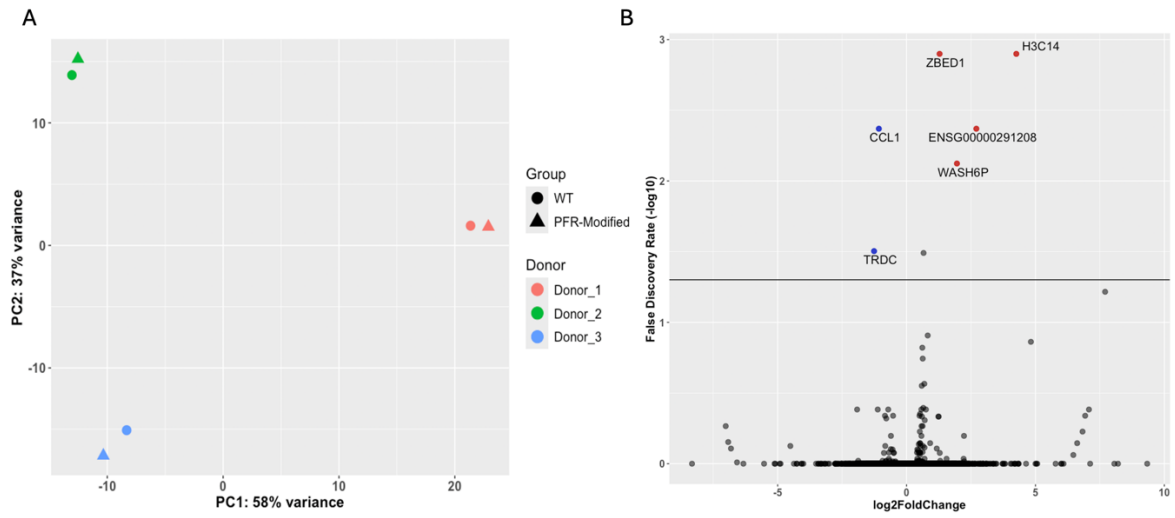


Supplementary Figure 4. Representative functional assays on P23 (DNAJB7$_{221-240}$)-specific & DNAJB7$_{241-260}$-specific T cell lines responding to relevant WT & variant peptides. A)

Representative IFN-γ ELISpot results using a P23 ($DNAJB7_{221-240}$)-specific T cell lines stimulated with T2-DR4 cells loaded with titrated concentrations of WT and variant peptides. (B) Dot plots representing TNF-α producing $CD4^+$ T cell lines specific to P23 (from HD02) cultured with $DNAJB7_{221-235}$ wild type peptides and modified variants-presented by T2-DR4 cells (gated on $CD4^+$ T cells). (C) Representative IFN-γ ELISpot results using a P25 ($DNAJB7_{241-260}$)-specific T cell lines stimulated with T2-DR4 cells loaded with titrated concentrations of WT and mutated peptides. (D) Representative dot plot showed intracellular cytokine responses from the P25 T cell line (from HC11) were TNF-α dominant (T2-DR4 cells were used as APC, cells gated on $CD4^+$).



Supplementary Figure 5. Cross over response of T cells expanded on P11 variant peptides derived from DNAJB7 and 5T4. (A) Summary of CD4+ T cell responses of 8 healthy controls and 12 CRC HLA-DR4+ donors expanded on WT or *P*11-modifed peptides. (B) Representative IFN-γ ELISpot results of response of short-term T cells lines, from a HLA-DR1+ healthy control (left) and a CRC patient (right), cultured with WT or *P*11R modified flank of $5T4_{111-130}$, then tested against either WT peptide or *P*11R variant peptides.



Supplementary Figure 6. RNA-sequencing analysis of CD4⁺ T cells restimulated with WT or PFR-modified peptides. (A) Principal component analysis (PCA) of variance stabilizing transformation-normalized gene expression data (top 500 most variable genes) from CD4⁺ T cells expanded and restimulated with either WT (circles) or PFR-modified (triangles) DNAJB7 (Donor_1 and Donor_2) and 5T4 (Donor_3 only) peptides. Samples cluster primarily by donor indicating donor-specific transcriptional variation as the main source of variability. (B) Volcano plot showing differentially expressed genes (DEGs) between WT DNAJB7 (n =2) and PFR-modified DNAJB7 group (n = 2) across two donors (Donor_1 and Donor_2). The x-axis represents $\log_2$ fold change ($\log_2$FC), and the y-axis shows the $-\log_{10}$ adjusted p-value (false discovery rate). A total of 6 genes (labelled) were significantly differentially expressed (padj < 0.05, absolute $\log_2$FC > 1), including 4 genes upregulated (red) and 2 genes downregulated (blue) in the PFR-modified condition. Upregulated: HC314: histone acetylation, ZBED1: cell proliferation; WASH6P: actin, tubulin binding; down regulated CCL1: secreted by activated T cells and displays chemotactic activity for monocytes and Tregs (via CCR8); TRDC: T cell receptor delta constant.